Analyses of IgE-epitope profiles from legume allergens as approach towards the development of novel diagnostic and therapeutic reagents in food allergy

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International and national presentations

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Popp J., Trendelenburg V., Niggemann B., Randow S., Völker E., Schwaben L., Pfeiffer L., Reuter A., Schiller D., Vieths S., Beyer K., Holzhauser T. Relevance of the major pea (*Pisum sativum*) allergen Pis s 1 and its IgE-epitope profile in pea-allergic versus sensitized tolerant children. Paul-Ehrlich-Institut Retreat on Biomedical Research, Ronneburg, Germany. January 16–18, 2017. **Oral presentation**.

Nürnberg J., Trendelenburg V., Niggemann B., Randow S., Reuter A., Schiller D., Vieths S., Beyer K., Holzhauser T. Recognition of linear IgE-binding peptides of Pis s 1 in pea-allergic versus tolerant children. European Academy of Allergy and Clinical Immunology Congress, Vienna, Austria. June 11–15, 2016. **Poster presentation (awarded Abstract Prize).**

Nürnberg J., Beyer K., Randow S., Kühne Y., Reuter A., Vieths S., Schiller D., Holzhauser T. Analyses of IgE-epitope profiles from legume allergens as approach towards the development of novel diagnostic reagents in food allergy. Paul-Ehrlich-Institut Retreat on Biomedical Research, Ronneburg, Germany. February 10–12, 2016. **Poster presentation**.

Table of contents

List of figures

List of tables

Abbreviations

°C	degree Celsius
AA	amino acid(s)
Api g	Nomenclature for allergens of *Apium graveolens*
APS	ammonium persulfate
Ara h	Nomenclature for allergens of *Arachis hypogaea*
AUC	area under curve
BCA	bicinchoninic acid
Bet v	Nomenclature for allergens of *Betula verrucosa*
BMGY	buffered glycerol-complex medium
BMMY	buffered methanol-complex medium
Boc	tert-butyloxycarbonyl
BSA	bovine serum albumin
CD	circular dichroism
cDNA	complementary DNA
Da	Dalton
DIC	N,N′-diisopropylcarbodiimide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DBPCFC	double-blind placebo-controlled food challenge
dH_2O	distilled water
DLS	dynamic light scattering
dNTP	deoxynucleotide
DTT	dithiothreitol
E. coli	*Escherichia coli*
EC_{50}	half maximal effective concentration
EDTA	ethylenediaminetetraacetic acid
e.g.	exempli gratia; for example
ELISA	enzyme-linked immunosorbent assay
EPIT	epicutaneous immunotherapy
et al.	et alia; and others
FcεRI	high-affinity IgE receptor
Fmoc	9-fluorenylmethyloxycarbonyl
g	gram

Gly m	Nomenclature for allergens of *Glycine max*
h	hour(s)
HCl	hydrochloric acid
$His_{(6)}$-tag	hexahistidine tag
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HRP	horseradish peroxidase
IgE	immunoglobulin E
IgG	immunoglobulin G
IL	interleukin
IMAC	immobilized metal ion affinity chromatography
IPTG	isopropyl-β-D-thiogalactopyranoside
IUIS	International Union of Immunological Societies
k	kilo
KCl	potassium chloride
KH_2PO_4	potassium dihydrogen phosphate,
l	litre
LB	lysogeny broth
LOD	limit of detection
LOQ	limit of quantification
M	molar
mA	miliampere
Mal d	Nomenclature for allergens of *Malus domestica*
μF	microfarad
μg	microgram
min	minute(s)
μl	microliter
mM	minimolar
MOPS	3-(N-morpholino)propanesulfonic acid
MS	mass spectrometry
Mut^+	methanol utilization plus
Mut^S	methanol utilization slow
MWCO	molecular weight cut-off
NaCl	sodium chloride
Na_2HPO_4	disodium hydrogen phosphate

NaH_2PO_4	sodium dihydrogen phosphate
n.d.	not determined
NH_4HCO_3	ammonium bicarbonate
no.	number
OD	optical density
OIT	oral immunotherapy
OtBu	tert-butyl ester
p	pico
p.a.	pro analysis
PA1	pea albumin 1
PA2	pea albumin 2
PAGE	polyacrylamide gel electrophoresis
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
pdb	protein data bank
PEI	Paul-Ehrlich-Institut
Pis s	Nomenclature for allergens of *Pisum sativum*
PMSF	phenylmethylsulfonyl fluoride
P. pastoris	*Pichia pastoris*
Pru p	Nomenclature for allergens of *Prunus persica*
r	recombinant
r/a	reduced and alkylated
RBL	rat basophilic leukemia
ROC	receiver operating characteristic
rpm	revolutions per minute
RT	room temperature
SCIT	subcutaneous immunotherapy
SDS	sodium dodecyl sulfate
SEC	size exclusion chromatography
SIT	specific immunotherapy
SLIT	sublingual immunotherapy
SOC	super optimal broth
TAE	Tris-acetate-EDTA
TBS	Tris-buffered saline

tBu	tert-butyl ether
TEMED	N,N,N',N'-tetramethylethylenediamine
Tris	tris(hydroxymethyl)aminomethane
Trt	trityl
U	units
UV	Ultraviolet
v	volume
V	volt
w	weight
xg	multiples of the gravitational constant
YPD	yeast extract peptone dextrose medium
YPDS	yeast extract peptone dextrose medium with sorbitol

One letter code	**Three letter code**	**Amino acid**
A	Ala	Alanine
C	Cys	Cysteine
D	Asp	Aspartic acid
E	Glu	Glutamic acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine

1 Abstract

1.1 Zusammenfassung

Hülsenfrüchte (Leguminosen) sind eine wichtige Nahrungsquelle für hochwertige Proteine und pflanzliche Öle. Jedoch sind sie auch als Auslöser schwerer allergischer Reaktionen bekannt. Allergische Reaktionen vom Soforttyp werden durch allergene Proteine (Allergene) ausgelöst, vermittelt durch deren Interaktion mit dem Immunsystem über allergenspezifische Antikörper vom Typ Immunglobulin E (IgE). Um eine allergische Sensibilisierung zu diagnostizieren, können diese spezifischen IgE-Antikörper gegen Gesamtproteinextrakt des allergenen Lebensmittels, wie z.B. Erdnuss, *in vitro* bestimmt werden. Eine Sensibilisierung gegen Gesamtproteinextrakt ist jedoch nicht zwangsläufig ein Beleg für eine klinisch relevante Allergie. Um die diagnostische Spezifität zu erhöhen, wurde in den letzten Jahren vermehrt das spezifische IgE gegen einzelne Allergenkomponenten bestimmt. In diesem Zusammenhang wird z.B. das 2S Albumin aus Erdnuss, Ara h 2, als diagnostischer Marker für eine klinisch relevante Erdnussallergie beschrieben. Demnach könnten spezifische IgE-Bindungsmuster auf Protein- und Peptidebene als Ansatzpunkt für die Entwicklung neuer diagnostischer, aber auch therapeutischer Werkzeuge dienen. Jedoch ist die Relevanz allergener Speicherproteine, wie den 2S Albuminen und den 7S Globulinen, aus Erdnuss, Erbse und Soja teils unbekannt oder wird kontrovers diskutiert. Gleiches gilt für deren IgE-Bindungsstellen.

Daher war das Ziel dieser Arbeit, die IgE-Bindung von Leguminosenallergikern und Patienten, welche eine Leguminosensensibilisierung aufweisen, aber klinisch tolerant sind, zu vergleichen. Hierfür wurde die IgE-Bindung der allergischen und toleranten Patienten an homologe 2S- und 7S-Leguminosenallergene aus Erdnuss, Erbse und Soja auf Protein- und Peptidebene bestimmt. In diese Arbeit wurden Seren von allergischen und sensibilisierten, aber toleranten, Kindern mit einem Leguminosenextrakt spezifischem IgE $\geq$ 0.35 kU_A/L eingeschlossen. Für die Untersuchungen wurden jeweils Gesamtproteinextrakte und rekombinante 7S Globuline und 2S Albumine aus Erdnuss, Erbse und Soja hergestellt. Anschließend wurde in Immunoblot-Analysen die IgE-Bindung an die Extrakte und die rekombinanten Leguminosenallergene bestimmt und das spezifische IgE densitometrisch quantifiziert. Zur Untersuchung der IgE-Bindung auf Peptidebene wurden überlappende Peptide (15 Aminosäuren lang, 4 Aminosäuren Überlappung) aller untersuchten Leguminosenallergene synthetisiert und mittels CelluSpot™-Multipeptidmikroarrays gegen die Patientenseren getestet. Zusätzlich wurde auf Peptidebene der Einfluss der posttranslationalen Prolinhydroxylierung von Ara h 2 aus Erdnuss auf dessen IgE-Bindungsfähigkeit und auf die diagnostische Genauigkeit untersucht. Für Erdnuss- und Erbsenallergie konnten, gemäß der in dieser Studie festgelegten Selektionskriterien, spezifische diagnostische Kandidatenpeptide identifiziert werden. Anschließend wurde mittels ROC-

Kurvenanalyse (Receiver Operating Characteristic) der diagnostische Vorhersagewert der einzelnen rekombinanten Allergene und der spezifischen Kandidatenpeptide bestimmt und miteinander verglichen. Dadurch sollte bestimmt werden, ob Peptide als zusätzliche oder alternative Reagenzien in der in-vitro-Allergiediagnostik eingesetzt werden können. Aufgrund der sehr begrenzten Anzahl an verfügbaren und eingeschlossenen Sojaallergikern wurde Soja jedoch von einer detaillierten Peptid- und ROC-Kurvenanalyse ausgeschlossen.

Basierend auf den Immunoblot-Analysen und den jeweiligen Nachweisgrenzen zeigten 48%, 79% und 50% der Erdnuss-, Erbsen- und Sojaallergiker jeweils eine Serum IgE-Bindung an die 7S Globuline rAra h 1, rPis s 1 und rGly m 5.03. Im Gegensatz dazu zeigten von den gegen Erdnuss, Erbse und Soja sensibilisierten, aber klinisch toleranten Kindern jeweils nur 8%, 20% und 20% eine IgE-Bindung an die entsprechenden 7S Globuline.

Der auffälligste Unterschied zwischen den drei Leguminosen zeigte sich jedoch in Bezug auf die Relevanz der 2S Albumine. In der Immunoblot-Analyse zeigten jeweils 65% und 78% der erdnussallergischen Kinder eine IgE-Bindung an rAra h 2.01 bzw. rAra h 2.02. Im Gegensatz dazu zeigten die gegen Erdnuss sensibilisierten, aber klinisch toleranten Kinder keinerlei Bindung an die beiden Ara h 2 Isoformen. Diese Entdeckung unterstreicht die Relevanz und den diagnostische Wert der beiden Isoformen, insbesondere von Ara h 2.02, in der Vorhersage einer klinisch relevanten Erdnussallergie. Solch eine klinische Relevanz der 2S Albumine konnte in Erbse und Soja, basierend auf den Immunoblot-Analysen, nicht nachgewiesen werden. Hier zeigte sich eine vernachlässigbare oder fehlende IgE-Bindung der erbsen- und sojaallergischen Kinder an die jeweils untersuchten 2S Albumine. Eine ROC-Kurvenanalyse der mittels Immunoblot untersuchten rekombinanten Proteine ergab, dass rAra h 2.02 (AUC (Area under curve) 0.86) und rPis s 1 (AUC 0.86) den höchsten diagnostischen Vorhersagewert für eine klinisch relevante Erdnuss- bzw. Erbsenallergie besaßen, auch im Vergleich zum jeweiligen Leguminosenextrakt.

Im Hinblick auf potenzielle diagnostische Peptide konnten bei Ara h 2 zwei Peptidpaare (AUC 0.87-0.90) mit einem diagnostischen Vorhersagewert identifiziert werden, welcher mit dem des Volllängenproteins rAra h 2.02 (AUC 0.86) vergleichbar war. Jedes der beiden Peptidpaare enthielt mindestens ein Peptid mit hydroxylierten Prolinresten. Darüber hinaus konnte für Peptide von Ara h 2 beobachtet werden, dass die Hydroxylierung von Prolinresten die Sensitivität bei gleichbleibender Spezifität erhöht, was diese posttranslationale Modifikation zu einem interessanten Ziel für zukünftige diagnostische Ansätze macht. Für eine mögliche in-vitro-Diagnostik der Erbsenallergie konnten elf diagnostische Peptide von Pis s 1 identifiziert werden, welche mit einer AUC von 0.99, verglichen mit Erbsenextrakt (AUC 0.81) und rPis s 1 (AUC 0.86), den höchsten diagnostischen Vorhersagewert in dieser Studienpopulation hatten.

Zusammengefasst waren in dieser pädiatrischen Studienpopulation signifikante Unterschiede hinsichtlich der allergenen Potenz der 2S und 7S Speicherproteine in den drei untersuchten Hülsenfrüchten erkennbar. In Erdnuss konnten Ara h 1 und Ara h 2 als relevante Allergene identifiziert werden. Jedoch zeigte sich, basierend auf den Immunoblot-Analysen, dass das 2S Albumin Ara h 2 und insbesondere die Isoform Ara h 2.02 das relevanteste Allergen mit dem höchsten diagnostischen Vorhersagewert in dieser Studienpopulation war. Im Gegensatz dazu konnten in dieser Studie die 2S Albumine aus Erbse und Soja als relevante Allergene ausgeschlossen werden. Wohingegen das 7S Globulin Pis s 1 als Haupt- und immunodominantes Allergen in Erbse identifiziert werden konnte. Ein ähnlicher Trend schien sich bei Gly m 5.03 aus Soja abzuzeichnen. Aber aufgrund der sehr begrenzten Anzahl an eingeschlossenen sojaallergischen Kindern, ließen sich keine weiteren Schlussfolgerungen ziehen.

Darüber hinaus ergab die Untersuchung der IgE-Bindung auf Peptidebene, dass Peptide mit einem diagnostischen Vorhersagewert identifiziert werden konnten, welcher vergleichbar oder sogar besser als der der jeweiligen rekombinanten Volllängenproteine war. Diese Peptide können möglicherweise als zusätzliche oder alternative Reagenzien in der in-vitro-Allergiediagnostik dienen. Dies erfordert jedoch zunächst eine weitere Überprüfung in einer prospektiven Studie.

Außerdem wurden auf der Grundlage dieser Arbeit neue Erkenntnisse über relevante Allergene und deren IgE-Bindungsstellen gewonnen, welche die Entwicklung neuartiger therapeutischer Reagenzien mit verbesserten Eigenschaften unterstützen können. In diesem Zusammenhang wurde die IgE-Bindung an lineare Peptide in Annäherung an lineare IgE-Epitope untersucht. Anhand eines 27-mer Peptids, welches hydroxylierte Prolinreste enthielt, konnte eindeutig gezeigt werden, dass lineare IgE-bindende Epitope von natürlichem Ara h 2 eine relevante Mastzelldegranulation induzieren können. Dieses Resultat unterstreicht die potenzielle Relevanz linearer IgE-Bindungsstellen bezüglich der Gesamtallergenität und sollte daher bei der Entwicklung neuer immuntherapeutischer Ansätze zur Behandlung von Erdnussallergien berücksichtigt werden, da andernfalls Patienten einem höheren Risiko unerwünschter Nebenwirkungen ausgesetzt sein könnten. Darüber hinaus können die in dieser Studie identifizierten immunodominanten IgE-Bindungsstellen von Pis s 1 als Grundlage für die Entwicklung von hypoallergenen Substitutionsvarianten für die allergenspezifische Immuntherapie von Erbsenallergien dienen. Die Relevanz dieser Ergebnisse sollte in Bezug auf Wirksamkeit und Sicherheit in präklinischen Studien zur Therapie von Erdnuss- bzw. Erbsenallergie weiter untersucht werden.

1.2 Abstract

Legumes are an important source of high-value proteins and edible oils in the human diet. However, they are also a known and frequent source of severe allergic reactions to foods. Immediate-type allergic reactions are elicited by allergenic proteins (allergens), mediated by their interaction with the immune system via allergen-specific antibodies of the isotype immunoglobulin E (IgE). These specific IgE antibodies to total protein of the allergenic food, e.g. peanut, can be measured *in vitro* to diagnose an allergic sensitization. While specific IgE to total protein extracts indicates a sensitization, it is not necessarily a proof for clinical reactivity. In contrast, the measurement of specific IgE to selected allergen components, such as the peanut 2S albumin Ara h 2, has been proposed to improve the *in vitro* diagnostic specificity for a clinical reactivity. In this context, specific recognition patterns of IgE binding at the protein level, and further at the peptide level, may allow the development of advanced diagnostic approaches, and innovative therapeutic reagents. However, knowledge about the relevance of allergenic storage proteins, such as 2S albumins and 7S globulins, of the legumes peanut, pea and soybean and their IgE-binding peptides is in part controversial or limited.

Therefore, this study aimed to determine the serum IgE binding of legume-allergic versus sensitized but clinically tolerant children to homologous 2S and 7S legume allergens from peanut, pea and soybean at the protein and peptide level.

In this study, sera from legume-allergic as well as sensitized but clinically tolerant children with legume extract-specific IgE $\geq$ 0.35 kU_A/L were included. Total protein extracts and recombinant 7S globulins and 2S albumins of peanut, pea, and soybean, respectively, were prepared. Afterwards, serum IgE binding to legume extracts and recombinant legume allergens was individually determined by means of densitometric immunoblot analysis. Overlapping peptides (15 AA; offset 4 AA) representing the full-length allergens were synthesized and analyzed for their ability to bind serum IgE on CelluSpot™ multipeptide microarrays. The influence of post-translational hydroxylation of proline residues in peanut Ara h 2 on the capacity to bind serum IgE and thus on the diagnostic value was additionally investigated on the peptide level. Potential candidate diagnostic peptides specific for peanut and pea allergy were identified, according to predefined selection criteria that were established in this study. Finally, the diagnostic value of the investigated recombinant legume allergens and of the potential candidate diagnostic peptides was determined as area under curve (AUC) by receiver operating characteristic (ROC) curve analysis. By doing so, it should be determined whether peptides may serve as additional or alternative reagents in the *in vitro* diagnosis of legume allergy. However, due to the very limited number of available and included soybean-allergic patients, soybean was excluded from a detailed analysis of candidate diagnostic peptides and ROC curve analysis.

According to immunoblot analysis and the specific limit of detection, serum IgE of 48%, 79% and 50% of peanut-, pea- and soybean-allergic children bound to the 7S globulins rAra h 1, rPis s 1 and rGly m 5.03, respectively. Of the peanut-, pea- and soybean-sensitized but tolerant children 8%, 20% and 20% showed a serum IgE binding to the respective 7S globulins.

The most striking difference between the three legumes could be observed regarding the relevance of the 2S albumins. In immunoblot analysis, 65% and 78% of peanut-allergic children showed a serum IgE binding to rAra h 2.01 and rAra h 2.02, respectively. In contrast, serum IgE of peanut-sensitized but tolerant patients did not bind to any of both Ara h 2 isoforms. Hence, the relevance and the diagnostic value of both 2S albumin isoforms, especially of rAra h 2.02, in peanut allergy were further highlighted. Based on immunoblot analyses, such clinical relevance of 2S albumins could not be found in pea and soybean. Here, negligible or no serum IgE binding to 2S albumins was detectable in pea- as well as soybean-allergic children. Based on the ROC curve analysis of the investigated recombinant proteins analyzed in immunoblot, rAra h 2.02 (AUC 0.86) and rPis s 1 (AUC 0.86) had the highest diagnostic value in peanut and pea allergy, respectively, also compared to the respective total legume protein extract.

With regard to potential diagnostic peptides, two Ara h 2-derived peptide pairs (AUC 0.87-0.90) with a diagnostic value comparable to that of full-length rAra h 2.02 (AUC 0.86) could be identified for peanut allergy. Each of the two peptide pairs contains at least one peptide with hydroxylated proline residues. In addition, it could be observed that hydroxylation of proline residues in Ara h 2-derived peptides increased the sensitivity while maintaining the specificity which makes this post-translational modification an interesting target for future diagnostic approaches. Moreover, for pea allergy eleven candidate diagnostic peptides of Pis s 1 could be identified. Compared to pea extract and rPis s 1, the candidate peptides had, with an AUC of 0.99, the highest diagnostic value in this pea study population.

In this pediatric study population, general differences in allergenic potency of the 2S and 7S storage proteins of the three investigated legumes were apparent. In peanut, Ara h 1 and Ara h 2 could be identified as relevant allergens. However, based on immunoblot analysis, the 2S albumin Ara h 2, in particular the isoform Ara h 2.02, was the most relevant allergen with the highest diagnostic value in this peanut study population. In contrast, in pea and soybean 2S albumins were excluded as relevant allergens in this study. In pea, the 7S globulin Pis s 1 was identified as a major and immunodominant allergen. A similar trend seemed to be observed for Gly m 5.03 and soybean, but due to the limited number of soybean-allergic patients included in this study, no further conclusions can be drawn.

In addition, the investigation of IgE binding at the linear peptide level revealed that peptides with a diagnostic value that is comparable or even better than that of the respective full-length recombinant proteins could be identified. These peptides may potentially serve as additional or alternative reagents in the *in vitro* diagnosis of legume allergy; however, this requires verification in a prospective study.

Moreover, based on this study, additional knowledge about relevant allergens and their IgE-binding sites was obtained. This knowledge can support the development of novel therapeutic reagents with improved characteristics. In this context, IgE binding to short peptides of allergens was investigated to localize IgE-binding sites in approximation to linear (continuous) IgE epitopes. It could be shown that linear IgE-binding epitopes of natural Ara h 2, as represented by a 27-mer peptide, still induced relevant mast cell degranulation. This finding underpins the potential relevance of linear IgE-binding sites with regard to total allergenic potency, and should thus be considered in the development of novel reagents for the immunotherapeutic treatment of peanut allergy as otherwise patients might be at high risk of unintended side effects. In addition, identified immunodominant IgE-binding sites of Pis s 1 may allow the development of hypoallergenic substitution variants for allergen-specific immunotherapy of pea allergy. The relevance of these findings with regard to efficacy and safety can be further addressed in preclinical models of peanut and pea allergy, respectively.

2 Introduction

2.1 Pathomechanism of type I allergic reaction

With an increasing prevalence, approximately 20-30% of the population suffer worldwide from allergic diseases making them a public health concern (Pawankar et al. 2011).

In 1906, the pediatrician Clemens von Pirquet firstly described the term 'allergy' as a change in reactivity of the immune system comprising similarly hyper- and hyposensitivity reactions (Huber 2006). Today the term 'allergy' describes an immunological hypersensitivity reaction against harmless, non-infectious environmental proteins, called allergens (Galli et al. 2008; Johansson et al. 2004). Known allergen sources are, for example, pollen, animal dander, house dust mite, food, latex and insect venoms (Galli et al. 2008).

According to the Gell-Coombs classification hypersensitivity reactions are divided into four types: the IgE-mediated immediate reaction (type I), the antibody-mediated cytotoxic reaction (type II), the immune-complex-mediated reaction (type III) and the cell-mediated reaction (type IV) (Descotes and Choquet-Kastylevsky 2001).

IgE-mediated type I allergic reactions (Figure 1), including food allergy, are divided into a sensitization phase followed by the immediate phase, in which the patient shows the typical allergic symptoms (Larché et al. 2006). The sensitization phase is initiated by the internalization and the processing of allergens by antigen-presenting cells such as dendritic cells which afterwards present resulting allergen-derived peptides on major histocompatibility complex (MHC) class II molecules on their surface to naïve T cells (Galli et al. 2008; Holgate and Polosa 2008). The recognition of the peptide MHC class II complex by the T-cell receptor (TCR) on naïve T cells and the interaction of co-stimulatory molecules located on antigen-presenting cells and naïve T cells leads to an activation and differentiation of the respective naïve T cell (Humeniuk et al. 2017). Depending on the surrounding cytokines, T cells can differentiate into T helper 1 (Th1), Th2, Th17 and induced regulatory T (iTreg) cells (Zhu and Paul 2008). In the case of type I allergic reactions T cells differentiate into Th2 cells (Humeniuk et al. 2017). Naïve B cells interact with the native allergen through their B cell receptor and load it on their surface via MHC class II molecule (Poulsen and Hummelshoj 2007). Activated B cells ligate with Th2 cells via CD40-CD40L and CD80/86-CD28 interactions (Galli et al. 2008; Poulsen and Hummelshoj 2007). These interactions in combination with the presence of cytokines such as IL-4 induce the differentiation of the activated B cell into an allergen-specific IgE-producing plasma B cell. Allergen-specific IgE antibodies bind to mast cells and basophil granulocytes by their high-affinity IgE receptor (FcεRI) (Poulsen and Hummelshoj 2007). The production of allergen-specific IgE antibodies and their binding to mast cells and basophils constitute the sensitization phase. It is noteworthy that the presence of allergen-specific IgE antibodies does

not necessarily mean that a patient is allergic, it only indicates that a sensitization took place (Poulsen and Hummelshoj 2007).

The immediate phase of the allergic reaction is characterized by the cross-linking of FcεRI-bound IgE on mast cells and basophils after repeated allergen exposure (Larché et al. 2006; Poulsen and Hummelshoj 2007). This requires the presence of at least two binding sites (epitopes) for allergen-specific IgE on the surface of allergens. In general, linear and conformational epitopes can be distinguished. Linear epitopes are displayed by a continuous stretch of the amino acid sequence of the allergen. In contrast, conformational epitopes consist of amino acids that are discontinuous in the primary structure but folding of the allergen brings the amino acids into spatial proximity (Pomés 2010). The cross-linking of FcεRI-bound IgE then induces the degranulation and the release of vasoactive amines (mainly histamine), lipid mediators (prostaglandins and cysteinyl leukotrienes), chemokines and other cytokines (Larché et al. 2006; Poulsen and Hummelshoj 2007). Finally, the release of these mediators causes immediate allergic symptoms such as allergic rhinitis, asthma, urticaria, gastrointestinal reactions (vomiting and diarrhea) and anaphylaxis (Galli et al. 2008).

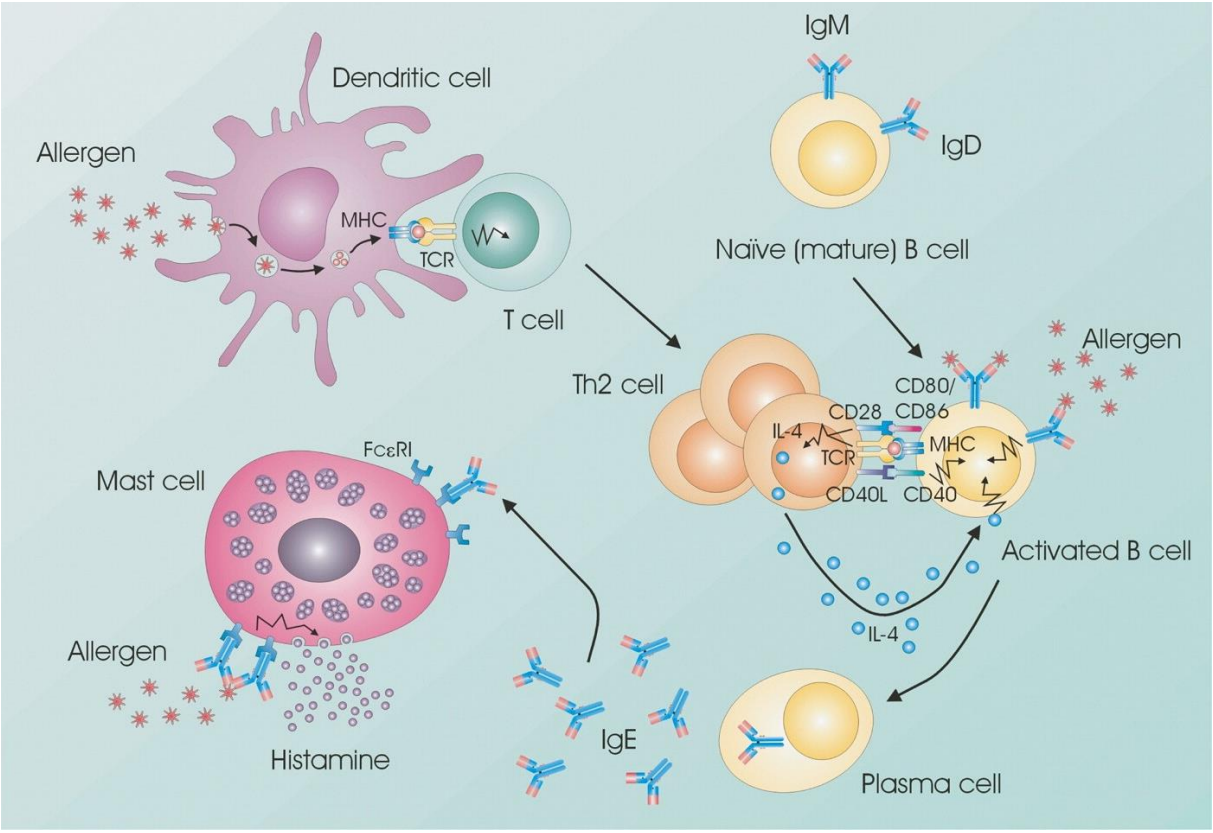


Figure 1: Pathomechanism of IgE-mediated type I allergic reaction (adapted from (Poulsen and Hummelshoj 2007)).

The reasons why some people develop allergies compared to others remaining tolerant are still unclear. Several host (e.g. heredity) and environmental factors (e.g. exposure to allergens) are suggested being responsible for the development of an allergy. Furthermore, reduced infections in childhood may increase the risk of developing an allergic disease (Swert 1999).

Several properties are described that can explain the allergenicity of a protein but so far no general paradigm is developed for the prediction of a protein's allergenicity. Structural, physicochemical and biochemical properties or aspects like functional domains, hydrophobicity, stability, formation of oligomers or aggregates, solubility and post-translational modifications (e.g. hydroxylation) are described as contributing to the allergenicity of a protein (Bernard et al. 2015; Scheurer et al. 2015). Furthermore, with increasing evidence allergens are attributed to possess intrinsic adjuvant properties (e.g. lipid- and carbohydrate binding properties, and protease activity) stimulating the innate immunity (Scheurer et al. 2015).

2.2 IgE-mediated food allergy

Precise data on the prevalence of food allergy are lacking but published data suggest that, depended on the investigated food, up to 10% can be affected, with food allergies being more common in children compared to adults (Sicherer and Sampson 2018). However, the accurate determination of food allergy prevalence is hindered by influencing factors such as geographic variation, study populations and methodologies (Sicherer and Sampson 2014). For example, the prevalence of self-reported food allergy is compared to the prevalence defined by objective methods like oral food challenges of lower evidence (Rona et al. 2007).

Depending on the clinical appearance, the route of allergic sensitization and the underlying immunologic mechanisms, IgE-mediated food allergies can be divided into class 1 (classic) and class 2 (pollen-associated) food allergies (Breiteneder and Ebner 2000; Valenta et al. 2015). In class 1, also known as classic food allergy, the sensitization occurs via the gastrointestinal tract and eliciting allergens are described as being resistant to gastric digestion. The most important class 1 food allergens are cow's milk, hen's egg and legumes. Class 1 food allergy affects more frequently young children, whereas class 2 food allergy is more frequently found in adults. Class 2 or pollen-associated food allergy is caused by IgE cross-reactivity between sensitizing inhalant allergens (e.g. birch pollen Bet v 1) and homologous nonsensitizing but allergic reaction eliciting food allergens (e.g. apple Mal d 1, celery Api g 1) (Breiteneder and Ebner 2000). Compared to class 1 food allergens, stability of class 2 food allergens is considered to be more affected by heat and proteolytic digestion. Therefore, class 2 food allergens may likely not cause a sensitization via the gastrointestinal tract but instead via the respiratory tract (Egger et al. 2006; Valenta et al. 2015). However, this is also controversially discussed.

Food allergy can lead to severe allergic reactions and especially legumes are described as the most common foods triggering anaphylaxis. A more detailed analysis showed age-dependent trigger profiles with hen's egg, cow's milk and peanut being the most frequently elicitors in children, and wheat, celery, soy and shellfish in adults (Worm et al. 2014).

According to structural and biological properties, animal and plant food allergens can be classified into families and superfamilies. The majority of animal food allergens belong to one of three major families, the caseins, tropomyosins and EF-hand proteins (Mills 2011).

Plant food allergens can be classified into four common protein families and superfamilies: the cupin superfamily (7S and 11S globulin seed storage proteins), the prolamin superfamily (2S albumin seed storage proteins, nonspecific lipid-transfer proteins, cereal α-amylase and protease inhibitors, and cereal prolamins), the proteins of the plant defense system (pathogenesis-related proteins, Kunitz-type protease inhibitors and proteases) and the profilins (Breiteneder and Radauer 2004).

Seed storage proteins are known food allergens and a sensitization to legume seed storage proteins, such as peanut Ara h 1, Ara h 2 and Ara h 3 or soybean Gly m 5 and Gly m 6, is considered as an indication for an increased risk of severe allergic reactions (Asarnoj et al. 2010; Holzhauser et al. 2009; Vereda et al. 2011).

2.2.1 Legume seed storage proteins

Legumes belong to the Fabaceae family and include many allergenic foods, for example, peanut, soybean, lentil, lupine, chickpea and pea. Due to their high protein and lipid content, legumes have a high nutritional value and are thus preferably included in the human diet (Verma et al. 2013).

More than 80% of total legume seed protein is constituted by the seed storage proteins (Kigel and Galili 1995). They serve as a source of amino acids and nitrogen during germination and seedling growth. Despite different structures, seed storage proteins share common properties. Their expression is tissue-specific and dependent on the stage of development. In the mature seed, seed storage proteins form discrete compartments called protein bodies. Seed storage proteins are commonly secretory proteins containing an N-terminal signal peptide which is co-translationally cleaved as the protein is translocated from the cytosol into the lumen of the endoplasmic reticulum. In the lumen of the endoplasmic reticulum, post-translational modifications like folding, disulfide bond formation, subunit assembly and glycosylation take place. Depending on their solubility and their sedimentation coefficients, legume seed storage proteins can be classified into three groups: 2S albumins, 7S and 11S globulins (Shewry et al. 1995).

2S albumins are small water-soluble proteins and although 2S albumins, like the other storage proteins, exhibit a high level of polymorphism they share common characteristics (Shewry et al. 1995). Typical 2S albumins are composed of a large and a small subunit linked by two disulfide bonds (Moreno and Clemente 2008). There are, however, some variations regarding subunit and disulfide bond formation. For example, peanut Ara h 2 is composed of a single chain. Whereas pea albumin 1 (PA1) is so far described as being composed of two separate subunits not linked by disulfide bonds (Higgins et al. 1986; Lehmann et al. 2006). 2S albumins have in common to contain five α-helices, a C-terminal loop and a solvent-exposed and flexible hypervariable region. The hypervariable region of 2S albumins is described as an important region containing immunodominant IgE epitopes (Moreno and Clemente 2008). Moreover, the whole protein is stabilized by four disulfide bonds that are formed between eight conserved cysteine residues (Moreno and Clemente 2008). The disulfide bonds result in the formation of a stable core structure that is highly resistant to proteolytic digestion and heating, another common characteristic of 2S albumins (Lehmann et al. 2006). Despite the above-mentioned common properties of 2S albumins, they share only a low sequence homology of 14 to 40% (Moreno and Clemente 2008).

The globulin storage proteins are salt-soluble proteins that can be divided on the basis of their sedimentation coefficients into 7S vicilin-type globulins and 11S legumin-type globulins (Shewry et al. 1995). Both storage proteins are bicupins characterized by two β-barrel core domains and belong to the cupin superfamily (Breiteneder and Radauer 2004).

7S globulins are trimeric proteins with molecular weights of 150 to 190 kDa. The subunits have molecular weights between 40 and 80 kDa and their composition varies considerably among different legume species due to different degrees of post-translational modifications such as proteolytic processing and/or glycosylation (Breiteneder and Radauer 2004; Shewry et al. 1995). Pis s 1 from pea, for example, undergoes post-translational proteolysis leading to subunits of different molecular weights (Gatehouse et al. 1982). Compared to 2S albumins, 7S globulins do not contain cysteine residues leading to the absence of disulfide bonds (Shewry et al. 1995).

The stabile structure of the trimeric complex of the 7S globulins is suggested being responsible for the relative resistance to heating and digestion. This stability is a property they have in common with the 2S albumins (Deshpande and Damodaran 1989; Maleki et al. 2000a).

11S globulins are hexameric proteins with each subunit being composed of an acidic and a basic chain associated by one interchain disulfide bond (Shewry et al. 1995). Mature hexameric 11S globulins have molecular weights between 300 and 400 kDa and are generally not glycosylated (Fukushima 1991).

It should also be pointed out that cross-reactivity between different legume species is a frequent immunological phenomenon (Verma et al. 2013). Cross-reactivity is the binding of antibodies directed against a specific allergen to other homologous proteins. High sequence identity and structural homologies are often the cause of cross-reactivity (Aalberse 2000). This cross-reaction can be of clinical relevance. For example, in peanut-allergic patients it could be shown that a clinically relevant cross-reactivity with lupine occurs frequently (Moneret-Vautrin et al. 1999). On the contrary, it is reported that a serological cross-reactivity between peanut and other legumes, which occurs frequently, is in less than 5% of clinical relevance (Sicherer and Wood 2012). Different dietary habits of the respective study population may be one reason explaining this controversy.

2.2.2 Diagnosis

An accurate allergy diagnosis is important to avoid unnecessary dietary restrictions and allergic reactions. The first steps in the diagnostic process are often a detailed analysis of the medical history and a physical examination. Both approaches should help identifying the offending food allergens and to differentiate the reaction from nonallergic disorders (Boyce et al. 2010). However, food allergies based on the reports of parents and patients need further verification as they tend to be overestimated (Rona et al. 2007). Moreover, hidden allergens or contaminated foods can influence the patient's history and lead to an incorrect allergy diagnosis (Ebo and Stevens 2001). An expert panel from the United States agrees that a diagnosis based alone on either medical history or physical examination is not sufficient sensitive and specific (Boyce et al. 2010). As add-on to a convincing history, sometimes an elimination diet avoiding the suspected offending food is recommended. Clearing allergic symptoms during elimination diet of a particular food can support the previous allergy diagnosis. However, elimination diets can lead to malnutrition and should therefore not be carried out over a longer period of time (Boyce et al. 2010).

Once the history is obtained and an IgE-mediated food allergy is suspected, skin prick tests (SPT) are often performed for further evaluation. During SPT food allergen extracts or fresh foods are pricked under the skin of the forearm or upper back of the patient with a lancet (Muraro et al. 2014). The test is considered positive when the patient shows a wheal at the prick site that is at least 3 mm larger than the wheal of the negative control. Accordingly, a positive SPT indicates a sensitization meaning that the patient has specific IgE antibodies to the tested food. Cross-linking of these IgE antibodies bound to cutaneous mast cells by food allergens induces wheal development (Boyce et al. 2010). The SPT is easy to handle, safe and a useful method to determine whether a patient is sensitized or not. A negative SPT result

excludes with a high predictive accuracy a clinically relevant food allergy (Sampson 2004). A positive SPT result, on the other hand, must be interpreted with caution as a sensitization does not necessarily mean that a patient is allergic and shows clinical symptoms. Which means that the SPT cannot differentiate between clinically relevant sensitization, leading to allergy, and irrelevant sensitization, i.e. tolerance. Moreover, cross-reactive proteins within different foods can lead to false-positive test results further hindering an accurate allergy diagnosis (Sicherer 2001). This illustrates that by using an extract-based SPT a differentiation between a co-sensitization and a cross-reactivity is not possible and is especially a problem when multiple positive test results are observed (Ferreira et al. 2014).

Another frequently used diagnostic method is the *in vitro* quantitative measurement of specific IgE antibodies to food extracts or to single allergen components in patients' blood samples (Renz et al. 2018). Very common in this context is the ImmunoCAP™ system (Thermo Fisher Scientific). The ImmunoCAP™ is an automated system using a cellulose matrix as solid phase to which the allergens are covalently bound. Allergen-bound serum IgE is afterwards detected by a fluorescently labeled anti-human IgE. sIgE levels are quantified by means of a World Health Organization (WHO) reference used for standard curve generation (Hamilton 2010). For some foods, like peanut, egg, milk and fish, 95% predictive food-specific IgE levels were established (Sampson and Ho 1997). However, there are some patients with sIgE levels below the specific cut-off/decision point but still showing allergic symptoms (Lopes de Oliveira et al. 2013). Furthermore, it could be shown that specific decision points vary depended on, for example, the used test system, patients' ages and the population (Komata et al. 2007; Perry et al. 2004; Wang et al. 2008). Like the SPT, the quantitative measurement of food extract-specific IgE poses the same problems to physicians: a positive result just indicates a sensitization and not necessarily a clinically relevant allergy. In addition, extracts contain different proteins that can be responsible for the allergic reaction on one side, but on the other side contain cross-reactive proteins binding serum IgE that can result in clinically irrelevant sIgE levels (Renz et al. 2018). Furthermore, missing or underrepresented as well as instable allergens within commercial extracts can lead to false-negative sensitization results (Muraro et al. 2014). The mentioned limitations of an extract-based diagnosis gave rise to a more sensitive and specific diagnostic approach, called component-resolved diagnostics (CRD), that measures the specific IgE to single allergen components. Allergen components used for CRD are either natural, purified allergens or recombinant allergens. Measurement of sIgE can be done via the above-mentioned ImmunoCAP™ system or via ImmunoCAP™ ISAC (Immuno-Solid phase Allergen Chip, Thermo Fisher Scientific). The ISAC is a multiplex platform where the sIgE in a low quantity of serum to more than 100 natural or recombinant allergen components can be measured in a single

assay in parallel (Canonica et al. 2013). A major advantage of CRD over extract-based testing is that it may enable a differentiation between genuine sensitization and cross-reactivity (Canonica et al. 2013; Ferreira et al. 2014). In some study populations, the use of food-specific marker allergens allows i) the identification of patients at high risk for developing an allergic reaction and ii) the prediction of the severity of the allergic reaction. For example, Beyer *et al*. identified sIgE to Ara h 2 and Cor a 14 as risk markers for peanut and hazelnut allergy, respectively (Beyer et al. 2015). A study from Asarnoj *et al.* analyzing a Swedish birth cohort suggested that a sensitization to the storage proteins Ara h 1, Ara h 2 or Ara h 3 is predictive for severe peanut allergy, whereas a sensitization to the birch-related protein Ara h 8 and birch pollen is predictive for mild peanut symptoms, if any (Asarnoj et al. 2010). This observation was further verified by an add-on study showing that a sensitization only to Ara h 8 is a strong indication for peanut tolerance (Asarnoj et al. 2012). Another study from Vereda *et al.* showed comparable results regarding the correlation between the sensitization profiles and the severity of the reaction, but also illustrates the limitations of this component-resolved approach. Depending on the geographical region, different component sensitization profiles could be observed. US patients were primarily sensitized to Ara h 1, Ara h 2 and Ara h 3, Spanish patients to Ara h 9 (nonspecific lipid-transfer protein) and Swedish patients to Ara h 8 (Vereda et al. 2011). Another limitation of CRD is that most of the used components are recombinant proteins representing just one isoform compared to their natural counterpart. The usage of a single recombinant isoform in CRD, however, can lead to a lower test sensitivity as some isoforms are less allergenic. Comparable with the other mentioned diagnostic approaches, CRD suffers from the same limitation as, except for a few examples, based on the presence or absence of sIgE to allergen components, the clinical phenotype cannot clearly be predicted in all cases. Nevertheless, CRD is a useful diagnostic approach but further studies are needed to establish and confirm correlations between sensitization profiles and clinical phenotypes in different study populations.

For the future, an epitope-resolved diagnosis analyzing the IgE binding on the epitope level instead of on the protein level is under investigation. By doing so, it might be possible to identify homologous IgE-binding areas on different allergens that might explain cross-reactivity, or to identify different IgE-binding areas that might explain a divergent allergic phenotype. Several studies suggest an additional diagnostic value of epitope information over extract and component-resolved diagnosis. Studies from Beyer *et al*. and Shreffler *et al.* on peanut allergy showed that the recognition of specific epitopes correlates with the clinical reactivity and that the IgE epitope diversity correlates with the severity of an allergic reaction, respectively (Beyer et al. 2003; Shreffler et al. 2004). Similar results could be observed for milk allergy where

higher IgE epitope diversity and affinity correlated with the clinical phenotype and the severity of the reaction (Wang et al. 2010). Another study from Lin *et al.* combined peptide microarray data with bioinformatic analyses to predict peanut allergy with an improved and high accuracy (Lin et al. 2012). However, at this time these studies require further validation before applied in clinical routine. Therefore, despite the many diagnostic efforts that have been made, the double-blind, placebo-controlled food challenge (DBPCFC) remains the gold standard in allergy diagnosis (Boyce et al. 2010). Here, increasing doses of the suspected offending food blinded in a matrix are administered to the patients and symptoms are recorded (Renz et al. 2018). Compared to the single-blind and the open food challenge, the DBPCFC is the least subjective and is least influenced by patient and physician bias (Boyce et al. 2010). As neither the patient nor the physician knows whether the placebo or the allergenic food is offered to the patient. To date, DBPCFC shows the highest specificity in the diagnosis of food allergy. However, it is time consuming, expensive and harbors the risk of severe allergic reactions limiting its use in routine clinical application (Boyce et al. 2010).

Thus, alternative diagnostic methods such as the above-mentioned promising epitope analysis reducing the need for DBPCFCs should be explored for their potential value in allergy diagnosis.

2.2.3 Treatment

Currently no causative therapy is available for food allergies in clinical routine and allergen avoidance is the only effective method of preventing an allergic reaction (Renz et al. 2018). However, allergen avoidance is often complicated by unlabeled food allergens (hidden allergens). Shared equipment during the manufacture of foods can lead to allergen cross-contamination and is therefore often the source of hidden allergens (Röder et al. 2008). Further, an extensive and longterm allergen avoidance can lead to nutritional deficiencies and affect quality of life (Muraro et al. 2014). For treatment of severe allergic symptoms adrenaline is administered, whereas local mild symptoms are treated with antihistamines (Renz et al. 2018). Currently, several allergen-specific and non-specific therapy strategies are under investigation. To date, due to safety and efficacy issues allergen-specific immunotherapies for food allergies are only administered in clinical trials (Muraro et al. 2014; Renz et al. 2018).

During allergen-specific immunotherapy (SIT) increasing doses of the offending food allergen are administered to patients with the goal to induce persistent tolerance (Jutel and Akdis 2011). Although SIT has been used for a longer time for the treatment of venom or inhalant allergies, its exact underlying mechanism is not yet fully understood. Several mechanisms effecting T- and B-cells are described. SIT induces the generation of regulatory T-cells suppressing Th2 cells and thereby the release of inflammatory Th2 cytokines (Jutel and Akdis 2011). Regulatory T-

cells secret IL-10 and TGF-β, two anti-inflammatory cytokines that suppress effector cells like mast cells, eosinophils and basophils of the allergic inflammation (Jutel and Akdis 2011; Jutel et al. 2013). Furthermore, regulatory T-cells and especially their secreted anti-inflammatory cytokine IL-10 suppress an inflammatory reaction on the B-cell level by suppressing IgE and by leading to an isotype switch from IgE to IgG4 (Jutel and Akdis 2011; Jutel et al. 2013; Meiler et al. 2008). IgG4 antibodies compete with IgE bound to mast cells for allergen binding and thereby inhibit mast cell and basophil degranulation which in turn leads to reduced allergic reactions (Jutel et al. 2013; Niederberger 2009).

Severe side effects during subcutaneous immunotherapy (SCIT) shifted the focus to oral immunotherapy (OIT). Several studies on peanut, egg or milk OIT report a successful desensitization in a subgroup of patients leading to a temporary tolerance immediately after the immunotherapy (Anagnostou et al. 2014; Caminiti et al. 2015; Pajno et al. 2010). However, only a limited number of desensitized patients develop a persistent tolerance where no regular allergen exposure is required to maintain protection (Caminiti et al. 2015). In addition, side effects ranging from mild to severe during OIT have led to the development of novel and safer therapeutic approaches (Anagnostou et al. 2014; Blumchen et al. 2010). These therapeutic approaches comprise, for example, other routes of application or the use of modified food allergens. Other routes of application include sublingual immunotherapy (SLIT) or epicutaneous immunotherapy (EPIT). A study comparing SLIT and OIT in peanut-allergic patients revealed that SLIT is safer but less efficacious (Narisety et al. 2015). Approaches combining SLIT with subsequent OIT showed promising results regarding efficacy, but nonetheless, oral administration caused in comparison to sublingual administration more systemic side effects (Keet et al. 2012). In EPIT, the allergen is administered to the skin via a patch. A study on EPIT showed a good safety profile, but similar to SLIT, OIT seems more efficacious (Jones et al. 2017).

Another approach to reduce the risk of side effects during immunotherapy and to increase efficacy is the use of modified allergens. In this context, for example, hypoallergens, synthetic peptides, plasmid DNA-based methods, conjugation to immunostimulatory sequences and chimeric allergen-human fusion proteins were investigated, as was summarized elsewhere (Cook and Burks 2018; Nowak-Węgrzyn and Sampson 2011). Allergens are modified by means of site-directed mutagenesis or chemical methods (e.g. reduction/alkylation) with the goal to generate hypoallergenic proteins that show a reduced IgE-binding capacity but a retained T-cell immunogenicity (Bannon et al. 2001; Swoboda et al. 2007; Toda et al. 2011; Zuidmeer-Jongejan et al. 2012). The use of single hypoallergens to treat food allergy can be hampered by allergies that are caused by multiple allergens. Furthermore, the use of unfolded allergens for

immunotherapy is not applicable for every food allergen. A study from Bernard *et al.* on peanut Ara h 2 showed that a sensitization to linear and conformational epitopes is dependent on the patient, highlighting that unfolded allergens can still be allergenic for some patients (Bernard et al. 2015).

Another approach using heat/phenol killed *Escherichia coli* expressing hypoallergenic peanut allergens Ara h 1, Ara h 2 and Ara h 3 modified by amino acid substitutions failed due to severe side effects (Wood et al. 2013).

Besides hypoallergens, allergen-derived peptides are also under investigation for their applicability in immunotherapy. Like hypoallergenic proteins, peptides aim at reducing side effects during immunotherapy while inducing immunologic tolerance. Therefore, short synthetic peptides containing allergen-derived T-cell epitopes are applied. The idea behind that approach is that peptides comprising between 8-16 amino acids are too short for cross-linking of FcεRI-bound IgE on effector cells and thus reduce the risk of an allergic reaction (Cook and Burks 2018). Simms *et al*. reported that an Ara h 1-derived peptide containing one T-cell epitope can protect against peanut-induced anaphylaxis in a mouse model (Simms et al. 2016). Further studies are required to investigate this in humans. Furthermore, the length of the administered peptides should be chosen carefully, as 15 AA long peptides can still induce a mediator-release *in vitro* (Bernard et al. 2015).

A further approach uses plasmid DNA encoding a major allergen to treat anaphylaxis in mice. Here, the oral administration of chitosan-plasmid DNA nanoparticles encoding Ara h 2 resulted in a modified immune system that protects against anaphylaxis (Roy et al. 1999).

The administration of allergens coupled to immunostimulatory sequences (e.g. CpG motives) represents another promising approach. Nanoparticles composed of protamine and CpG-oligonucleotides complexed with Ara h 2 were able to shift the immune system of mice toward Th1-cell response (Pali-Schöll et al. 2013). A further novel therapeutic strategy uses chimeric allergen-human fusion proteins. The idea behind this approach is that allergens fused to human IgG Fcγ1 can inhibit mast cell degranulation by an indirect cross-linking of FcεRI and FcγRIIb (Liu et al. 2013).

Beside allergen-specific, also non-specific strategies like anti-IgE therapy, traditional Chinese herbs, probiotics and cytokine/anticytokine therapies are in the focus of research (Nowak-Węgrzyn and Sampson 2011; Sicherer and Sampson 2009). Very promising in this context is the use of anti-IgE therapy in combination with OIT. Studies on peanut and cow's milk OIT showed that a treatment with omalizumab (humanized anti-IgE antibodies) allowed the administration of higher doses and reduced side effects, respectively (MacGinnitie et al. 2017; Wood et al. 2016).

2.3 Peanut allergy

Peanut allergy is one of the best-studied legume allergies so far and is especially associated with severe allergic reactions in children (Worm et al. 2014). Even small amounts like 0.2 mg peanut protein can elicit allergic reactions in 5% of peanut-allergic subjects (Taylor et al. 2014). Compared with other legume allergies, peanut allergy is reported to persist throughout life in most patients with only approximately 22% outgrowing it (Peters et al. 2015; Skolnick et al. 2001). According to a meta-analysis in Europe, the prevalence of food-challenged-defined peanut allergy is 0.2% (Nwaru et al. 2014).

Peanut has a total protein content of 21-29% which is comparable to pea but below the protein content of soybean (Dwivedi et al. 1990; Koppelman et al. 2001). However, peanut has with an average of 44% a high oil content (Dwivedi et al. 1990).

To date 16 peanut allergens (Ara h 1 to 17) and numerous isoforms are listed in the WHO/IUIS allergen nomenclature database (http://www.allergen.org/). These 16 allergens can be divided into the following families and superfamilies: cupins (Ara h 1, Ara h 3), prolamins (Ara h 2, Ara h 6, Ara h 7, Ara h 9, Ara h 16, Ara h 17), profilin (Ara h 5), Bet v 1-like (Ara h 8), glycosyl transferases GT-C (Ara h 10, Ara h 11, Ara h 14, Ara h 15) and scorpion toxin-like knottins (Ara h 12, Ara h 13) (Cabanillas et al. 2018; Mueller et al. 2014). Among these allergens, Ara h 1, Ara h 2 and Ara h 3 account together for the vast majority of the total peanut protein content and are together with Ara h 6 considered as the major peanut allergens (Hebling et al. 2012; Mueller et al. 2014). In particular, Ara h 2 is described in several publications as an important diagnostic marker for the prediction of peanut allergy (Beyer et al. 2015; Lieberman et al. 2013; van Erp et al. 2017).

Peanut, like most other legumes, is boiled or roasted before consumption. Therefore, the effect of thermal processing on its allergenicity has been widely studied with the result that roasting can lead to an increase in IgE-binding capacity while boiling decreases IgE-binding capacity of peanut extract (Maleki et al. 2000b; Mondoulet et al. 2005). However, the relevance of this finding with regard to clinical reactivity is still unclear.

2.3.1 Peanut allergen Ara h 1

Ara h 1, the first identified peanut allergen, is a well characterized vicilin-type 7S globulin with a molecular weight of ~64 kDa (Burks et al. 1991; Burks et al. 1995). It is a very abundant protein in peanut accounting for approximately 12-16% of the total peanut protein (Koppelman et al. 2001). Burks *et al.* identified two cDNA clones encoding Ara h 1, clone P41B and clone P17 with the sequence of P41B being registered in the WHO/IUIS database. Both sequences share high sequence homology and contain one N-linked glycosylation site and an N-terminal

signal peptide (Burks et al. 1995). Ara h 1 is described as a stable homotrimeric protein held together by hydrophobic interactions (Maleki et al. 2000a; Shin et al. 1998). Even roasting does not significantly affect the secondary structure of Ara h 1 and leads to an increased IgE-binding capacity and resistance to heating and digestion (Maleki et al. 2000b; Nesbit et al. 2012). Whereas boiling causes aggregation of Ara h 1 and resulted in a reduction in IgE binding (Blanc et al. 2011). The resolved crystal structure of the Ara h 1 core region shows high similarity with other known vicilin allergens like soybean Gly m 5 (Cabanos et al. 2011). In addition to the high structural similarity, Ara h 1 shows high sequence similarity with other vicilins like, for example, from lentil, pea and soybean, which additionally can cause IgE cross-reactivity (Barre et al. 2005; Kroghsbo et al. 2011).

Ara h 1 is a major allergen recognized by the great majority of peanut-allergic patients in most published studies (Burks et al. 1995; Kleber-Janke et al. 1999; Shreffler et al. 2004). A review focusing on the diagnostic accuracy of peanut components revealed for sIgE to Ara h 1, dependent on the study population and study design, sensitivities between 26-92% (median 56%) and specificities between 41-95% (median 85%). While the specificity of the 11S globulin Ara h 3 is comparable (median 86%), its sensitivity is lower (median 45%) (Klemans et al. 2015).

To date, 24 linear IgE epitopes of Ara h 1 have been identified, of which four are considered immunodominant as being recognized by more than 80% of peanut-allergic patients (Burks et al. 1997; Shreffler et al. 2004). It could be shown that the majority of the identified IgE epitopes of Ara h 1 are located at the monomer-monomer contact sites protected from degradation (Maleki et al. 2000a). Studies analyzing the allergenic potential of Ara h 1 digestion peptides revealed a significant IgE-binding capacity and an induction of mediator release in basophils (Eiwegger et al. 2006; Wichers et al. 2004).

Shreffler *et al.* observed a heterogeneous IgE binding to Ara h 1-derived peptides among peanut-allergic patients, but the authors could also show that the number of recognized peptides seems to correlate with the severity of the allergic reaction (Shreffler et al. 2004). Another study from Lin *et al.* analyzed peanut-allergic and peanut-tolerant patients for their IgE binding to peptides of peanut proteins. Amongst other results, an improvement in the diagnostic accuracy of Ara h 1 peptides compared to whole peanut extract could be shown (Lin et al. 2012).

2.3.2 Peanut allergen Ara h 2

Ara h 2, the second major allergen from peanut, is a monomeric 2S albumin seed storage protein with a molecular weight of ~18 kDa whose allergenic potential has been reported in several studies (Burks et al. 1992; Clarke et al. 1998; Kleber-Janke et al. 1999; Steven Stanley and

Bannon 1999). It accounts for 6-9% of the total peanut protein content and functions as trypsin inhibitor (Koppelman et al. 2001; Maleki et al. 2003). The allergen is composed of a single chain and as a member of the 2S albumins it contains the conserved eight cysteine residue motif forming 4 disulfide bonds (Lehmann et al. 2006). Another post-translational modification of Ara h 2 is the hydroxylation of specific proline residues (Li et al. 2010).

In general, Ara h 2 contains two isoforms, Ara h 2.01 (~17 kDa) and Ara h 2.02 (~18 kDa). Both share high sequence identity with the major difference being a 12 amino acid insertion in the isoform Ara 2.02 (Chatel et al. 2003). In addition, compared to Ara h 2.01, Ara h 2.02 contains three instead of two hydroxylated proline residues (Li et al. 2010).

Ara h 2 is a largely α-helical protein that shares high sequence and structure homology with Ara h 6 which in turn is responsible for the observed cross-reactivity of both peanut allergens (Lehmann et al. 2003; Lehmann et al. 2006). The structure of Ara h 2 and especially its core structure protects the protein from proteolytic digestion (Lehmann et al. 2006; Sen et al. 2002). It is reported that protease (trypsin and chymotrypsin) digested Ara h 2 still possess allergenic potency by inducing mediator release (Lehmann et al. 2006). In addition, Sen *et al.* reported that a 10 kDa protease-resistant fragment of Ara h 2 containing immunodominant IgE-epitopes can bind serum IgE from pooled peanut-sensitized patients (Sen et al. 2002).

Controversial data are available regarding the importance of linear and conformational IgE epitopes of Ara h 2 (Albrecht et al. 2009; Apostolovic et al. 2013; Bublin et al. 2013; King et al. 2005; Starkl et al. 2012). However, Bernard *et al.* convincingly showed that a sensitization to linear and conformational epitopes is dependent on the respective patient. The authors observed that in approximately half of the investigated peanut-allergic patients reduced and alkylated natural Ara h 2 and a 27-mer Ara h 2-derived peptide are still allergenic triggering mast cell degranulation (Bernard et al. 2015). In addition, the study by Starkl and co-workers showed a relevant remaining IgE binding and mediator release capacity of reduced and alkylated natural Ara h 2 (Starkl et al. 2012).

Furthermore, by comparing natural and recombinant Ara h 2 as well as Ara h 2-derived peptides with and without hydroxyproline residues, Bernard *et al.* highlighted the relevance of proline hydroxylation for proper IgE binding and allergenicity (Bernard et al. 2015). These observations highlight the importance of both types of epitopes and of post-translational hydroxylation as well as their consideration in the development of diagnostic and therapeutic approaches.

So far, more than ten IgE-binding epitopes of Ara h 2 have been identified and three of them suggested as being immunodominant. Two of the three immunodominant epitopes contain the amino acid sequence DPYSPS that is described as an important motif for IgE binding (Han et al. 2016; Stanley et al. 1997). This motif is present on a flexible surface loop and occurs two

and three times in Ara h 2.01 and Ara h 2.02, respectively (Chatel et al. 2003; Lehmann et al. 2006). Studies on both recombinant and natural Ara h 2 isoforms showed a higher IgE-binding capacity and assumed a higher allergenicity of Ara h 2.02 (Bernard et al. 2015; Chatel et al. 2003; Hales et al. 2004). Furthermore, Li *et al.* identified the second proline within this DPYSPS motif as being hydroxylated and Bernard *et al.* showed that this hydroxylation is relevant for the IgE-binding capacity and the allergenicity of Ara h 2 (Bernard et al. 2015; Li et al. 2010). In another experimental setup using liposomal nanoparticles, called nanoallergens, that display different linear IgE-binding epitopes of Ara h 2, similar results could be shown. Nanoallergens presenting a 15-mer peptide containing 2 repeated DPYSPS motifs with the second proline being hydroxylated showed, compared to the other analyzed epitopes and its counterpart without hydroxyproline, an enhanced immunogenicity triggering mast cell degranulation (Deak et al. 2017).

In general, Ara h 2 is attributed as high allergenic protein being a more potent elicitor in inducing mediator release in comparison to other peanut allergens (Blanc et al. 2009; Kulis et al. 2012; Palmer et al. 2005; Peeters et al. 2007).

The diagnostic value of sIgE to Ara h 2 in comparison to peanut extract and other peanut allergens has been widely investigated with the result that Ara h 2 shows the best accuracy in predicting peanut allergy in infants and children (Beyer et al. 2015; Dang et al. 2012; Ebisawa et al. 2012; Keet et al. 2013; Klemans et al. 2013b; Lieberman et al. 2013; Nicolaou et al. 2011; van Erp et al. 2017). In this context, the review of Klemans and co-workers reported for sIgE to Ara h 2 sensitivities ranging from 60% to 100% (median 88%) and specificities from 60% to 96% (median 85%) (Klemans et al. 2015).

Of all studies, Nicolaou *et al.* reported at a cut-off of 0.35 kU_A/L of Ara h 2 sIgE the highest sensitivity/specificity pair of 100% and 96%, respectively (Nicolaou et al. 2011). Areas under the receiver operating characteristic (ROC) curves (AUC) of 0.84-0.99 highlight an almost perfect accuracy in diagnosing peanut allergy (Beyer et al. 2015; Dang et al. 2012; Ebisawa et al. 2012; Klemans et al. 2013b; Lieberman et al. 2013; Nicolaou et al. 2011; van Erp et al. 2017). However, all published studies on the diagnostic value of sIgE to Ara h 2 are based on ImmunoCAP™ or ImmunoCAP™ ISAC measurements using *E. coli*-expressed recombinant Ara h 2.01 (personal communication Dr. Jonas Lidholm, Thermo Fisher Scientific and http://www.phadia.com/en/Products/Allergy-testing-products/ImmunoCAP-Allergen-Information/Food-of-Plant-Origin/Allergen-Components/rAra-h-1-recombinant-Peanut/). A potential increase in the diagnostic accuracy due to the use of Ara h 2.02 compared to Ara h 2.01 has not yet been shown.

Ara h 2.01-derived peptides are also under investigation with regard to their diagnostic application (Beyer et al. 2003; Lin et al. 2012; Otsu et al. 2015). Using SPOT-membrane peptide arrays displaying the three immunodominant epitopes of Ara h 2.01, formerly identified by Stanley *et al.*, and sera from peanut-allergic and peanut-sensitized but clinically tolerant patients, Beyer *et al.* could show that differences in IgE binding exist between both study groups. Between ~60-70% of peanut-allergic patients recognize each of the three immunodominant epitopes compared to less than 10% of peanut-tolerant patients (Beyer et al. 2003). In a more recent approach, Lin *et al.* used peptide microarray data and combined them with bioinformatic analyses to increase the accuracy of IgE-binding peptides in diagnosing peanut allergy. The authors determined serum IgE binding from peanut-allergic and tolerant patients to overlapping peptides covering the entire primary structures of Ara h 1, Ara h 2.01 and Ara h 3. Hereby, especially the IgE binding to peptides of Ara h 2.01 showed, in comparison to Ara h 1 and Ara h 3, the best discrimination between allergic and tolerant patients. By applying machine learning methods on microarray data, the authors could identify four peptide biomarkers increasing the diagnostic accuracy and reaching a sensitivity and specificity of 90% and 97%, respectively. These four peptides are each one peptide of Ara h 1 and Ara h 3 and two of Ara h 2.01 with the two Ara h 2.01 peptides being described as the key biomarkers (Lin et al. 2012).

Even though the mentioned studies show promising results, the isoform Ara h 2.02 and the post-translational hydroxylation of proline within the DPYSP^{OH}S motif have not been considered in any such study so far.

2.3.3 Identified gaps in peanut allergen knowledge

Ara h 1, Ara h 2 and Ara h 3 are considered as the major peanut allergens. Of these proteins, Ara h 2 has so far the highest diagnostic value, including high sensitivity and specificity. Compared to Ara h 2, Ara h 1 also has a high specificity but a lower sensitivity, whereas Ara h 3 has an even lower sensitivity (as outlined in chapters 2.3.1 and 2.3.2). Therefore, the 2S albumin Ara h 2 and the 7S globulin Ara h 1 were selected for further investigation.

Despite the fact that several studies are published on the relevance and the diagnostic value of Ara h 1, Ara h 2.01 and derived peptides, at the beginning of this PhD project, no diagnostic studies have been published that also take into account the second Ara h 2 isoform Ara h 2.02. Although there is evidence that Ara h 2.02 may be even more relevant than Ara h 2.01 and may possibly improve peanut allergy diagnosis (Bernard et al. 2015; Hales et al. 2004). Therefore, recombinant Ara h 1, Ara h 2.01, Ara h 2.02 and derived peptides should be analyzed for their relevance and diagnostic value using sera from symptomatic peanut-allergic versus tolerant

sensitized subjects without clinical symptoms. In addition, special attention should be given to peptides of Ara h 2.01 and Ara h 2.02 containing hydroxylated proline residues as the diagnostic and therapeutic relevance of the post-translational site-specific hydroxylation within the Ara h 2 binding sites has not been investigated at the beginning of this PhD project to the best of knowledge.

2.4 Pea allergy

Pea as well as chickpea and lentil play an increasingly important role as basic ingredients in the vegan diet. After boiling, these legumes are processed into different dishes like spreads, salads or real main dishes.

Allergy to pea (*Pisum sativum*) is with an estimated prevalence of 0.2% less common compared to other legume allergies (Kroghsbo et al. 2011). Some basic work on pea allergens and pea allergy has been done by Sanchez and co-workers (Sanchez-Monge et al. 2004). However, pea allergy has been less investigated than, for example, peanut allergy. Hence, only limited data are published on pea allergens.

Moreover, compared to soybean and peanut, pea has not been assigned as an ingredient for mandatory labeling in the EU (Regulation (EU) No 1169/2011). However, pea has been recognized as an emerging allergenic food (personal communication Prof. Dr. K. Beyer) and severe allergic reactions have been described in children and adults after ingestion of pea (Lavine and Ben-Shoshan 2019; Wensing et al. 2003). The unintended ingestion of hidden pea protein in food products was reported as the major cause of anaphylactic reactions in a recently published Canadian pediatric case series (Lavine and Ben-Shoshan 2019).

Moreover, relevant clinical cross-reactivity has been described with chickpea, lentil and peanut (Martínez San Ireneo et al. 2008; Wensing et al. 2003).

The protein content of pea seeds is on average 22% and the majority of extractable pea proteins are globulins with an average content of 70% of the total protein (Tzitzikas et al. 2006). So far, only three pea allergens, namely Pis s 1, Pis s 2 and Pis s 3, have been registered by the WHO/IUIS Allergen Nomenclature Sub-Committee (http://www.allergen.org/). Pis s 1 and Pis s 2 are globulin storage proteins and have been suggested by Sanchez and co-workers as potential major pea allergens. However, this study was essentially based on a serum pool and natural protein preparations enriched in vicilins. In addition, individual data on serum IgE binding were largely undisclosed (Sanchez-Monge et al. 2004).

Pis s 1 is a 44 kDa vicilin, whereas Pis s 2 is described as a 63 kDa convicilin (Croy et al. 1980; Sanchez-Monge et al. 2004). Both proteins share high sequence identity (~70%) and serological cross-reactivity, with the major difference being an N-terminal insertion in the

sequence of Pis s 2 (Bown et al. 1988; Croy et al. 1980). Pis s 3 belongs to the prolamin superfamily and is a 9.5 kDa non-specific lipid-transfer protein (nsLTP) that was recently identified and structurally characterized as a putative food allergen with IgE cross-reactivity to the nsLTP and major peach allergen Pru p 3 (Bogdanov et al. 2016).

Despite IgE cross-reactivity to Pis s 3, Bogdanov and co-workers just included patients with a sensitization to Pru p 3 and did not provide evidence for a pea allergy (Bogdanov et al. 2016). Compared to other legumes, such as peanut and soybean, no albumin has yet been described as an allergen in pea. However, it is reported that the albumin fraction might also include allergenic proteins (Malley et al. 1975; Sell et al. 2005).

2.4.1 Pea allergen Pis s 1

It is reported that Pis s 1 comprises between 1% and 8% of the total protein in pea extract. In this context, Kroghsbo *et al.* calculated a content of 1% to 4%, whereas Tzitzikas *et al.* reported that Pis s 1 accounts for 4% to 8% of the total pea protein content (Kroghsbo et al. 2011; Tzitzikas et al. 2006).

Pis s 1 is described as a glycosylated protein that undergoes extensive post-translational proteolysis leading to subunits ranging from 12.5 to 36 kDa (Badenoch-Jones et al. 1981; Gatehouse et al. 1981; Gatehouse et al. 1982; Sanchez-Monge et al. 2004). In contrast, Pis s 2 is not glycosylated and does not undergo such post-translational cleavage (Bown et al. 1988; Croy et al. 1980).

Published data on immunochemical properties of Pis s 1 are rare and are only based on purified vicilin fractions. Before Pis s 1 was identified as an allergen, a study by Wensing *et al.* detected clinically relevant cross-reactivity between pea and peanut with Ara h 1 and pea vicilin being responsible for this cross-reactivity. By investigating sera from three pea-allergic patients who subsequently developed peanut allergy, the authors could identify pea vicilin as the primary sensitizer in this study population using immunoblot as well as ELISA inhibition experiments with extracts, natural Ara h 1 and pea vicilin (Wensing et al. 2003). In 2004, Sanchez-Monge *et al.* identified Pis s 1 as potential major allergen from pea. In this study, sera from 18 pea-allergic patients were pooled or individually analyzed for their IgE binding to pea extract or pea extract fractions enriched in pea vicilin. The authors reported that 77% and 55% of the analyzed patients showed an IgE binding to full-length Pis s 1 and its proteolytic 32 kDa subunit, respectively. The other proteolytic subunits of Pis s 1 were also bound by serum IgE but to a lower extent (16-26%). Furthermore, the study revealed that Pis s 1 is heat stabile to boiling and cross-reacts with Len c 1 from lentil. Within this study the sequences of two isoforms of Pis s 1, namely Pis s 1.0101 and Pis s 1.0102, could be identified (Sanchez-Monge et al. 2004).

2.4.2 Putative pea allergens PA1 and PA2

The albumin fraction of pea contains two major components, the low molecular weight pea albumin 1 (PA1) and the high molecular weight pea albumin 2 (PA2) (Gruen et al. 1987). Although both proteins belong to the albumins, their suspected particular functions are different and their respective belonging to the conventional storage proteins is assumed being different. PA1 is a sulfur-rich protein that contributes ~50% of the total pea seed sulfur amino acids although it accounts for less than 10% of the pea seed total protein (Higgins et al. 1986). This in combination with its degradation upon germination suggests that it may function as sulfur storage protein (Gatehouse et al. 1985; Gruen et al. 1987; Higgins et al. 1986). The 11 kDa PA1 proprotein can be proteolytically cleaved into two mature proteins, PA1a (~6 kDa) and PA1b (~4 kDa). The described potential proteolytic cleavage of PA1 comprises the removal of a linker propeptide and a C-terminal propeptide composed of 6 and 8 amino acids, respectively. Unlike the conventional 2S albumin storage proteins, PA1a and PA1b are described as not being linked by interchain disulfide bonds, thus, potentially leading to two separate monomers (Higgins et al. 1986). Currently, six variants of PA1 (PA1 A-F) with only minor sequence variations are listed in the UniProt database (http://www.uniprot.org/) highlighting the multigenic character of this pea 2S albumin. More recent research on PA1b has shown that this protein possess insecticidal activity (Da Silva et al. 2010; Jouvensal et al. 2003).

The content of PA2 in pea seeds is comparable to PA1 but due to a lower content of sulfur amino acids, it accounts for only ~11% of the total pea seed sulfur amino acids (Higgins et al. 1987). In contrast to conventional storage proteins, PA2 is synthesized without signal peptide, is localized in the cytosol and is not significantly degraded in germinating seeds which seems to exclude a function as conventional storage protein (Croy et al. 1984; Higgins et al. 1987). Regarding the function of PA2, Vioque *et al.* suggested that it may function as a lectin and Vigeolas *et al.* observed a role of PA2 in polyamine metabolism (Vigeolas et al. 2008; Vioque et al. 1998). PA2 shows high sequence homology with a 2S albumin from lentil and is a homodimer (48-53 kDa) consisting of the two subunits PA2a (~25 kDa) and PA2b (~24 kDa) that show high sequence identity (Croy et al. 1984; Gruen et al. 1987; Higgins et al. 1987).

The relevance of both pea albumins, PA1 and PA2, as potential allergens in pea-allergic patients is still unclear and none of both is so far registered as an allergen. Beside two studies by Vioque *et al.*, who analyzed chickpea-sensitive individuals for their IgE binding to purified natural PA1 and PA2 in dot blot analysis, no IgE-binding data are currently available (Vioque et al. 1998; Vioque et al. 1999).

2.4.3 Identified gaps in pea allergen knowledge

At the beginning of this PhD project, allergy to pea has been little investigated at the clinical and molecular level compared to peanut. Due to the very limited data on the relevance of individual allergens, *in vitro* diagnosis of pea allergy, that complements clinical anamnesis, is based solely on pea extract allergen preparations. In contrast, a component-based approach may improve the diagnostic accuracy as outlined for peanut. With regard to the relevance of individual pea components, the 7S globulin Pis s 1 has been suggested as a potential major allergen. In addition, it is speculated that the albumin fraction, including the major components PA1 and PA2, may also contain allergenic proteins. However, detailed data on relevant or major albumin and vicilin allergens of pea and their IgE-binding sites are currently lacking.

Therefore, recombinant Pis s 1, PA1 and PA2 should be generated and investigated for their relevance and diagnostic value using sera from symptomatic pea-allergic versus pea-sensitized but clinically tolerant children. Moreover, derived peptides should be additionally investigated for their serum IgE-binding capacity and their diagnostic value.

2.5 Soybean allergy

The estimated prevalence of food-challenged-defined soybean allergy is 0.3% in Europe (Nwaru et al. 2014). It is reported that the majority (~50%) of soybean-allergic children outgrow their allergy and develop tolerance at the age of 7 years (Savage et al. 2010). Compared with the other two legumes, peanut and pea, soybean has with ~40% the highest protein content making it an even more important nutrient supplier (Liu 1997). The consumption of soybean products like soy milk, soy sauce, soy oil and tofu has increased in Europe over the last few years. Especially tofu is preferably included in the vegetarian diet as meat substitute. In addition, soy is used as emulsifier, protein filler and texturizer in the industrial food production. Many foods like breads, cakes, sausages and margarine contain soy proteins and thus harbor the risk of triggering an allergic reaction due to accidental consumption (Steinman 1996).

Several IgE-binding soybean proteins have been identified but only eight of them are registered as allergens in the WHO/IUIS database: the hullproteins Gly m 1 and Gly m 2, the birch pollen-related allergens Gly m 3 and Gly m 4, the storage globulins Gly m 5 and Gly m 6, the storage albumin Gly m 8 as well as the seed biotinylated protein Gly m 7 (Ballmer-Weber and Vieths 2008; Cabanillas et al. 2018; Holzhauser et al. 2009; Kattan et al. 2011; Riascos et al. 2016).

Especially the storage proteins are described as major soybean allergens in children (Ebisawa et al. 2013; Ito et al. 2011). However, the diagnostic value of soybean allergens is limited due to controversial results of independent studies (Ebisawa et al. 2013; Fukutomi et al. 2012; Holzhauser et al. 2009; Ito et al. 2011; Kattan and Sampson 2015; Klemans et al. 2013a; Lin et

al. 2006; Vissers et al. 2011). Differences in the study populations (e.g. age) or in the dietary habits due to geographic variations might explain the observed inconsistencies.

In addition, Maruyama and co-workers recently published the so far largest component-resolved diagnostic (CRD) study including all soybean allergen components. By investigating the serum IgE binding of Japanese children with suspected soybean allergy to soybean extract and individual components, the authors observed a patient-dependent, rather individual sensitization profile. Moreover, no single soybean component alone had a high diagnostic value in this Japanese cohort (Maruyama et al. 2018).

Currently, for serological soybean allergy diagnosis using ImmunoCAP™ or ImmunoCAP™ ISAC recombinant (r) and natural (n) allergen components rGly m 4, nGly m 5 and nGly m 6 are available (www.thermoscientific.com/phadia/de), in addition to soybean extract-based analysis.

2.5.1 Soybean allergen Gly m 5

Gly m 5, or β-conglycinin, is a vicilin-type 7S globulin that accounts for approximately 30% of the total seed proteins (Maruyama et al. 2001). The whole protein has a molecular weight of 150-200 kDa and is composed of the three subunits α, α' and β having molecular weights of 67 kDa, 71 kDa and 50 kDa, respectively (Maruyama et al. 1998). The three subunits α, α' and β are referred to as Gly m 5.0101, Gly m 5.0201 and Gly m 5.0301/Gly m 5.0302, respectively (Holzhauser et al. 2009). After their synthesis on the rough endoplasmic reticulum and the cotranslational processing including removal of signal peptide and N-glycosylation, the subunits assemble into trimers which are transported into the vacuole where the subunits are further processed to the mature densely packed trimeric from. Different trimeric subunit compositions are described including homotrimers. Despite N-terminal extension regions in α and α', all three subunits consist of a homologous core region that shows a sequence homology of ~70-90% (Maruyama et al. 1998). Furthermore, Gly m 5 shows sequence homology with other 7S globulins from hazelnut, pea and peanut leading to serological cross-reactivity (Kroghsbo et al. 2011).

One of the first studies analyzing the IgE-binding capacity of Gly m 5 was published by Holzhauser *et al.* in 2009. The authors analyzed soybean-allergic children and adults and could identify a sensitization to Gly m 5 in 43% of the study population, with all children being sensitized to Gly m 5 (Holzhauser et al. 2009). Furthermore, this study and a study from Japan by Ito *et al.*, who analyzed Japanese soybean-allergic children, revealed a correlation of sIgE to Gly m 5 and Gly m 6 with the severity of symptoms of soybean allergy (Holzhauser et al. 2009; Ito et al. 2011).

All three subunits of Gly m 5 can bind serum IgE from soybean-allergic patients (Holzhauser et al. 2009; Maruyama et al. 2018).

Depending on the investigated study population, different sensitization frequencies to the three subunits of Gly m 5 were reported. In previous unpublished investigations in the research group of Dr. Thomas Holzhauser (Division of Allergology, Paul-Ehrlich-Institut), sensitization to the the β-subunit Gly m 5.03 was more frequent in European soybean-allergic subjects (adults and children) than to the α or α' subunits of Gly m 5, namely Gly m 5.01 and Gly m 5.02. In contrast, a recently published CRD study from Japan reported the highest frequency of sensitization to the α-subunit Gly m 5.01 (92.5%) and only a 60% sensitization frequency to Gly m 5.03 in Japanese soy-allergic children (Maruyama et al. 2018).

Studies on adult soybean-allergic patients instead reported no predominant sensitization to Gly m 5 and Gly m 6 or no correlation of Gly m 5 and Gly m 6 with the severity of symptoms (Fukutomi et al. 2012; Klemans et al. 2013a; Vissers et al. 2011). Klemans *et al.* from the Netherlands detected a correlation of sIgE to Gly m 5 and Gly m 6 with rather mild and not severe allergic symptoms (Klemans et al. 2013a). Studies from Vissers *et al.* and Fukutomi *et al.* found a predominant sensitization to Gly m 4 and no or only a limited sensitization to Gly m 5 and Gly m 6 in adult soybean-allergic patients (Fukutomi et al. 2012; Vissers et al. 2011). The findings of these three studies show that the sensitization profiles are different between soybean-allergic adults and children. All three studies report a correlation between the sIgE to soybean Gly m 4 and birch pollen Bet v 1. This suggests that in adult soybean-allergic patients a sensitization via the gastrointestinal tract is rather rare and soybean allergy is probably mediated by IgE cross-reactivity between Bet v 1 and its homologue Gly m 4.

The diagnostic value of sIgE to Gly m 5 was assessed in comparison to other soybean components in soybean-allergic children from Japan, from the US and in soybean-allergic adults from the Netherlands, respectively, in three independent studies. All studies could detect a sensitization to Gly m 5 in their soybean-allergic study population; however in all three study populations the diagnostic value of Gly m 5 was below that of Gly m 8, the soybean 2S albumin, leading to the author's hypothesis that Gly m 8 might be a better marker for soybean allergy (Ebisawa et al. 2013; Kattan and Sampson 2015; Klemans et al. 2013a). However, in a larger CRD study, sensitization to Gly m 8 in Japanese children was only 60% (Maruyama et al. 2018). To date only IgE-binding peptides of Gly m 5.01 have been analyzed and identified, but the potential of IgE-binding peptides to improve soybean allergy diagnosis has never been investigated (Sun et al. 2013).

2.5.2 Soybean allergen Gly m 8

Two 2S albumins sharing 74% sequence identity could be isolated from soybean seeds: 2S albumin 1 (AL1) and 2S albumin 3 (AL3) (Lin et al. 2004). Since 2014, AL3 (UniProt accession no. P19594) is registered as an allergen in the IUIS database and is designated as Gly m 8.

The mature Gly m 8 is described as being composed of two subunits linked by two disulfide bonds (Lin et al. 2004). Furthermore, it is characterized as an α-helical protein, which is resistant to heating and chemical denaturation (Lin et al. 2004).

Regarding the relevance of this soybean allergen, controversial data are available. The first study on the IgE reactivity of Gly m 8 was published by Lin *et al.* in 2006. Using sera from European soybean-allergic patients with the majority being children, the authors could not detect any serum IgE binding to rAL1, rAL3 and nAL3 (Lin et al. 2006). Although Vissers *et al.* reported a higher level of sIgE to Gly m 8 compared to Gly m 5 and Gly m 6, the authors identified Gly m 4 as the protein with the highest predictive value in their study population. This study population was composed of adult soybean-allergic patients having a very low level of soybean-specific IgE but being primarily sensitized to Bet v 1 (Vissers et al. 2011). Other studies in contrast report a predominant role of sIgE to natural Gly m 8 (nGly m 8) in predicting soybean allergy (Ebisawa et al. 2013; Kattan and Sampson 2015; Klemans et al. 2013a). Using sera from Japanese children sensitized to soybean, ~90% of soybean-allergic children showed a sensitization to nGly m 8 (nAL3). ROC curve analysis in comparison to Gly m 5 (AUC 0.69) and Gly m 6 (AUC 0.64) showed a better diagnostic accuracy of Gly m 8 (AUC 0.75) in this Japanese cohort (Ebisawa et al. 2013). This result is in accordance with a study from Klemans *et al.* analyzing soybean-allergic and tolerant adults for their sensitization to Gly m 4, Gly m 5, Gly m 6 and Gly m 8. The authors from this Dutch study concluded, based on their ROC curve analyses, that Gly m 8 had the best diagnostic value in their study population, although the AUC of Gly m 8 (0.79) was comparable to Gly m 6 and soybean extract (both 0.77) (Klemans et al. 2013a). In addition, a study from the US on soybean-allergic and sensitized but tolerant children diagnosed by oral food challenge suggested Gly m 8 (AUC 0.82) as the best diagnostic marker for clinical soybean allergy (Kattan and Sampson 2015).

However, a recently published CRD study by Maruyama and co-workers, who analyzed Japanese soybean-allergic children for their sensitization to recombinant soybean components, reported lower AUC values of rGly m 8 (AUC 0.71) and the subunits of rGly m 5 (AUC 0.49-0.61). No single component alone had a high diagnostic value and therefore the authors established a novel approach by generating a fusion protein composed of Gly m 8 and the low cross-reactive N-terminal extension region of Gly m 5.02 (α' subunit of Gly m 5). By doing so,

the diagnostic accuracy could be increased in this Japanese study population (AUC 0.80) (Maruyama et al. 2018).

However, the diagnostic value of the soybean 2S albumin Gly m 8 (maximum AUC 0.82) is not as high as that of its peanut counterpart Ara h 2 (maximum AUC 0.99) (Nicolaou et al. 2011). Neither the single component Gly m 8 nor the Gly m 5-Gly m 8 fusion component showed such a high diagnostic accuracy as peanut Ara h 2.

2.5.3 Identified gaps in soybean allergen knowledge

Depending on the respective study population with regard to population age and geographical region, controversial data on the relevance of soybean components Gly m 5 and Gly m 8 have been published at the beginning of this PhD project. Therefore, recombinant Gly m 5 and Gly m 8 should be analyzed for their serum IgE binding and their diagnostic value. The subunit Gly m 5.03 was chosen for further investigations of Gly m 5 using serum samples from German subjects because it previously showed the highest IgE-binding frequency among all three Gly m 5 subunits in European soybean-allergic subjects (unpublished data, Paul-Ehrlich-Institut). In addition, the potential of IgE binding to peptides of Gly m 5.03 and Gly m 8 to improve soybean allergy diagnosis has not yet been investigated.

2.6 Aim of this PhD project

The determination of specific recognition patterns of IgE-binding at the protein and peptide level may result in novel and more accurate diagnostic approaches, as well as in safe and efficacious therapeutic reagents, as compared to traditional total protein extract-based diagnostic or therapeutic allergen preparations. 2S albumins and 7S globulins have been shown as important legume allergens. However, controversial or incomplete data exist, at least in part, about their relevance in pediatric legume-allergic populations.

Therefore, the aim of this PhD project was to compare legume-allergic and sensitized but clinically tolerant children regarding their IgE binding to 2S albumin and 7S globulin legume allergens from peanut, pea and soybean at the protein level. By doing so, the relevance and the diagnostic value of individual allergen components should be determined for each investigated legume. According to the mentioned gaps in legume allergy knowledge (chapter 2.3.3, 2.4.3 and 2.5.3), the following 7S globulins and 2S albumins were in the focus of this PhD project: Ara h 1 and both Ara h 2 isoforms (Ara h 2.01 and Ara h 2.02) from peanut, Pis s 1 and both major albumin components (PA1 and PA2) from pea, and Gly m 5.03 and Gly m 8 from soybean. Furthermore, IgE-binding peptides specific for the individual allergen should be investigated and analyzed for their diagnostic value for the respective legume allergy. The *in vitro* diagnostic

value of allergen-specific peptides should be compared to that of the full-length allergen components and to total protein extracts in order to evaluate their use as potential diagnostic reagents to differentiate between allergy and sensitization without clinical reactivity.

In order to determine the relevance and the diagnostic value of individual full-length allergens, they were heterologously expressed, purified and physicochemically and immunochemically characterized. As expression system, *Pichia pastoris* was favored as it enables a simple non-denaturing purification of recombinant proteins from its cell culture supernatant and further it enables post-translational modifications such as disulfide bond formation and folding. These properties are especially advantageous in the generation and purification of 2S albumins that form disulfide bonds. Due to comparability, 7S globulins should be also expressed using *Pichia pastoris*. In the case of problems during expression in *Pichia pastoris*, *Escherichia coli* should be used as an alternative expression system.

For peptide analysis, multipeptide microarray slides displaying synthetic overlapping peptides that represent the investigated full-length allergen were generated. Using individual patient sera testing, IgE-binding peptides in approximation to linear epitopes were identified. Conformational epitopes were not investigated because of the complexity of elucidation but there is awareness of the potential relevance. Diagnostic values of total protein extracts, purified recombinant allergens and selected allergen-specific peptides should be done statistically using receiver operating characteristic (ROC) curve analysis.

Finally, by comparing the outcome of the three legume projects, attention will be drawn to concordance or divergence between the different legume allergens in children. Furthermore, based on protein and epitope data, additional knowledge for the development of optimized, therapeutic approaches should be discussed, e.g. with regard to an improved safety profile.

3 Materials and Methods

3.1 Materials

3.1.1 Equipment

Table 1: Commonly used equipment.

Equipment	Company
Biofuge (Heraeus® 28RS)	Thermo Fisher Scientific, Waltham, MA, USA
Cell density meter (Biowave WPA CO 8000)	Biochrom Ltd., Cambridge, UK
Centrifuge (5804 R)	Eppendorf AG, Hamburg, Germany
Centrifuge (5415 R)	Eppendorf AG, Hamburg, Germany
Centrifuge (5414 C)	Eppendorf AG, Hamburg, Germany
Circular dichroism (CD) spectropolarimeter (J-810S)	JASCO Germany GmbH, Groß-Umstadt, Germany
Constant Systems cell disruptor (TS series)	Constant Systems Ltd., Daventry, UK
Diaphragm pump (ME 2 NT)	Vacuubrand GmbH & Co. KG, Wertheim, Germany
Freezer -80°C	New Brunswick Scientific, Edison, NJ, USA
Freezer -20°C (Premium No Frost)	Liebherr-International Deutschland GmbH, Biberach an der Riß, Germany
Fridge (FK 5440)	Liebherr-International Deutschland GmbH, Biberach an der Riß, Germany
Gene Pulser® II electroporation system	Bio-Rad Laboratories GmbH, München, Germany
Heating and drying oven (Heraeus® UT 6060)	Thermo Fisher Scientific, Waltham, MA, USA
HPLC Smartline System	KNAUER Wissenschaftliche Geräte GmbH, Berlin, Germany
Imager system Fusion FX	Vilber Lourmat Deutschland GmbH, Eberhardzell, Germany
Incubator (Heraeus® B6060)	Thermo Fisher Scientific, Waltham, MA, USA
Incubator shaker (New Brunswick™ Innova® 44)	Eppendorf AG, Hamburg, Germany
Intas Gel Jet Imager	INTAS Science Imaging Instruments GmbH, Göttingen, Germany
Microwave (MICRO-CHEF FM 1915)	Moulinex, Alençon, France
Mini-PROTEAN® 3 Cell	Bio-Rad Laboratories GmbH, München, Germany
Multifuge® (Heraeus® 3S-R)	Thermo Fisher Scientific, Waltham, MA, USA
MultiPep peptide synthesizer	Intavis AG, Köln, Germany
NanoPhotometer® (NP80)	Implen GmbH, München, Germany
Novex™ XCell SureLock™ Mini-Cell Electrophoresis System	Thermo Fisher Scientific, Waltham, MA, USA
Orbital shaker (Polymax 2040)	Heidolph Instruments GmbH & Co. KG, Schwabach, Germany
RAGE® (Rapid Agarose Gel Electrophoresis) device (RGX-60/RGX-100)	Cascade Biologics, Inc., Portland, USA
Savant Speed Vac®	Thermo Fisher Scientific, Waltham, MA, USA
Scanner (HP Scanjet 8300)	HP Inc., Palo Alto, CA, USA
Semi-dry blotter (Pegasus)	PHASE Gesell. für Phorese, Analytik und Separation mbH, Lübeck, Germany
Slide Spotting Robot	Intavis AG, Köln, Germany

SpectraMax 340PC microplate reader	Molecular Devices, Sunnyvale, USA
Standard Power Pack (P25/P25T)	Biometra, Göttingen, Germany
Thermal cycler (2720)	Applied Biosystems, Foster City, CA, USA
Thermomixer comfort (1.5 ml	Eppendorf AG, Hamburg, Germnay
Ultracentrifuge (Sorvall Evolution RC)	Thermo Fisher Scientific, Waltham, MA, USA
Universal grinder (M20)	IKA®-Werke GmbH & Co. KG, Staufen, Germany
Vortex mixer (REAX 2000)	Heidolph Instruments GmbH & Co. KG, Schwabach, Germany
X-ray film processor (CURIX 60)	Agfa HealthCare GmbH, Bonn, Germany
Zetasizer Nano-ZS	Malvern Panalytical GmbH, Kassel, Germany

3.1.2 Chemicals and reagents

If not stated otherwise, chemicals and reagents were used in pro analysis (p.a.) grade. Chemicals and reagents used for peptide synthesis were purchased with the addition "for peptide synthesis". All chemicals and reagents were provided from Agfa (Bonn, Germany), AppliChem (Darmstadt, Germany), Bio-Rad (München, Germany), Carl Roth (Karlsruhe, Germany), KPL (Gaithersburg, MD, USA), Merck (Darmstadt, Germany), Roche Diagnostics (Mannheim, Germany), Sigma-Aldrich (Steinheim, Germany), Thermo Fisher Scientific (Schwerte, Germany) and VWR (Darmstadt, Germany) respectively.

3.1.3 Buffers and solutions

Table 2: Composition of buffers and solutions.

Buffer/Solution	**Composition**
Alkylation solution (LC-MS^E)	260 mM iodacetamide 50 mM NH_4HCO_3
Anode buffer 1 (pH 10.4)	0.3 M tris 20% (v/v) methanol
Anode buffer 2 (pH 10.4)	25 mM tris 20% (v/v) methanol
Blocking buffer (immunoblot)	TBS pH 7.4 2% powdered milk
Blocking buffer (microarray)	10% blocking buffer (10x) (B6429; Sigma-Aldrich) 0.05% Tween20® 5% sucrose
Cathode buffer (pH 7.6)	40 mM 6-aminohexanoic acid 20% (v/v) methanol
CapMixture	4% (v/v) acetic anhydride 5% 2,6-lutidine 91% 1-methyl-2-pyrrolidinone
Cleavage solution	5% (v/v) dH_2O 2.5% (v/v) triisopropylsilane 88.5% (v/v) trifluoroacetic acid 4% trifluoromethanesulfonic acid

Coomassie staining solution	36% glacial acetic acid 28.5% methanol 0.2% (w/v) Coomassie Brilliant Blue G250
Coomassie destaining solution	10% glacial acetic acid 30% methanol
Denaturing buffer	20 mM sodium phosphate buffer pH 7.6 500 mM NaCl 20 mM imidazole 8 M urea per 500 ml buffer one protease inhibitor cocktail tablet (cOmplete Tablets, Mini, EDTA-free, Roche Diagnostics)
Destaining solution (LC-MS^E)	40% ethanol 50mM NH_4HCO_3
Dialysis buffer 1	20 mM sodium phosphate buffer pH 7.6 10 mM NaCl 1 mM EDTA
Dialysis buffer 2	20 mM sodium phosphate buffer pH 7.6 500 mM NaCl 1 mM EDTA
Elution buffer 1	20 mM sodium phosphate buffer pH 7.6 100 mM NaCl 1 mM EDTA 500 mM imidazole
Elution buffer 2	20 mM sodium phosphate buffer pH 7.6 500 mM NaCl 500 mM imidazole per 500 ml buffer one protease inhibitor cocktail tablet (cOmplete Tablets, Mini, EDTA-free, Roche Diagnostics)
Elution buffer (LC-MS^E)	25 mM NH_4HCO_3 10% acetonitrile
Incubation buffer (immunoblot)	TBS pH 7.4 0.05% Tween20® 2% powdered milk
Native PAGE running buffer (10x)	0.25 M tris 1.92 M glycine
Native PAGE sample buffer (5x)	0.31 M Tris-HCl pH 6.8 0.05% bromphenol blue 50% glyercol
Native PAGE separation gel (15%)	4.8 ml dH_2O 10 ml Rotiphorese® NF-Acrylamide/bis-solution 30% 5 ml Tris-HCl (1.5 M) pH 8.8 200 μl APS (10%) 20 μl TEMED
Native PAGE stacking gel (5%)	6.9 ml dH2O 1.7 ml Rotiphorese® NF-Acrylamide/bis-solution 30% 1.25 ml Tris-HCl (1 M) pH 6.8 100 μl APS (10%) 10 μl TEMED
PBS (pH 7.4)	137 mM NaCl 2.7 mM KCl 10 mM Na_2HPO_4 1.8 mM KH_2PO_4
Ponceau S solution	0.1% (w/v) Ponceau S 5% glacial acetic acid
Reduction solution (LC-MS^E)	65 mM DTT 50 mM NH_4HCO_3

Renaturing buffer	20 mM sodium phosphate buffer pH 7.6 500 mM NaCl 20 mM imidazole per 500 ml buffer one protease inhibitor cocktail tablet (cOmplete Tablets, Mini, EDTA-free, Roche Diagnostics)
SDS-PAGE running buffer (10x)	0.25 M tris 1.92 M glycine 1% (w/v) SDS
SDS-PAGE sample buffer (5x)	10% (w/v) SDS 0.1% (w/v) bromphenol blue 0.25 M Tris-HCl pH 6.8 50% glycerol 7.7% (w/v) DTT
SDS-PAGE separation gel (15%)	4.6 ml dH_2O 10 ml Rotiphorese® NF-Acrylamide/bis-solution 30% 5 ml Tris-HCl (1.5 M) pH 8.8 200 μl SDS (10%) 200 μl APS (10%) 20 μl TEMED
SDS-PAGE stacking gel (5%)	6.8 ml dH_2O 1.7 ml Rotiphorese® NF-Acrylamide/bis-solution 30% 1.25 ml Tris-HCl (1 M) pH 6.8 100 μl SDS (10%) 100 μl APS (10%) 10 μl TEMED
Side-chain cleavage solution	5% (v/v) dH_2O 3% (v/v) triisopropylsilane 12% (v/v) dichloromethane 80% (v/v) trifluoroacetic acid
Sodium phosphate buffer (0.1 M, pH 7.6)	84.5 ml Na_2HPO_4 (1 M) 15.5 ml NaH_2PO_4 (1 M) ad 1000 ml dH_2O
TAE buffer	40 mM tris 20 mM acetic acid 1 mM EDTA
TBS (pH 7.4)	50 mM tris 150 mM NaCl 50 mM HCl (37%)
Size exclusion buffer	20 mM sodium phosphate buffer pH 7.6 10 mM NaCl 1 mM EDTA
Washing buffer (IMAC)	20 mM sodium phosphate buffer pH 7.6 100 mM NaCl
Washing buffer (immunoblot/microarray)	TBS pH 7.4 0.05% Tween20®

3.1.4 Cell culture media

Table 3: Media composition.

Medium	Composition
BMGY medium	1% yeast extract 2% peptone 100 mM potassium phosphate, pH 6.0 1.34% yeast nitrogen base (without amino acids) 4 x 10^{-5}% biotin 1% glycerol
BMMY medium	1% yeast extract 2% peptone 100 mM potassium phosphate, pH 6.0 1.34% yeast nitrogen base (without amino acids) 4 x 10^{-5}% biotin 0.5% methanol
LB medium (pH 7.0)	1% tryptone 1% yeast extract 0.5% NaCl
LB agar	LB medium 1.5% agar-agar
RBL medium	MEM (Mimimal Essential Medium) 5% [v/v] fetal calf serum (FCS) 1% [v/v] L-glutamine
YPD medium	1% yeast extract 2% peptone 2% dextrose
YPDS medium	1% yeast extract 2% peptone 2% dextrose 1 M sorbitol
YPDS agar	YPDS medium 1.5% agar-agar

3.1.5 Specific antibodies and secondary conjugates

Table 4: Antibodies and secondary conjugates.

Antibody/Secondary conjugate	Clone	Company
Mouse anti-human IgE Fc-HRP	B3102E8	SouthernBiotech via Biozol, Eching, Germany
Mouse anti-$(His)_6$-tag	13/45/31	DIANOVA GmbH, Hamburg, Germany
Mouse anti-Ara h 1 (PN-t)	unknown	Dr. Wolf-Meinhard Becker, Forschungszentrum Borstel, Germany
Goat anti-mouse IgG (γ-chain specific)-HRP	polyclonal	Sigma-Aldrich, Steinheim, Germany
Streptavidin-HRP	not applicable	SouthernBiotech via Biozol, Eching, Germany

3.1.6 Patient sera

In the peanut, pea and soybean project 35, 19 and 21 children were included (Table 14, 24 and 32), respectively. Inclusion was based on a peanut-, pea- and soybean-specific IgE level ≥ 0.35 kU_A/L, respectively. According to oral food challenge or convincing history, patients were either allergic to the respective legume or clinically tolerant. Patient serum samples were collected at the Charité Universitätmedizin Berlin and local ethics committees authorized donations. In this PhD thesis the following serum overlaps between the three legume projects were: peanut patient 30 corresponded to soybean patient 13, peanut patient 12 equaled soybean patient 9, and pea patient 8 equaled soybean patient 1. Detailed information on patient characteristics are given in Table 14, 24 and 32. In immunoblot analyses, serum from a non-allergic subject (N) served as negative control and was used for calculating the respective limit of detection (LOD). For standard curve generation for densitometric quantifications of specific IgE levels in immunoblot analyses, serum RS14239 (103.61 kU_A/L sIgE to Ara h 1), serum RS14231 (54.1 kU_A/L sIgE to Ara h 2) or serum PEI163 (27.32 kU_A/L sIgE to Gly m 5) were used. In addition, in the pea project serum PEI131 (22.1 kU_A/L sIgE to peach extract (ImmunoCAP™ f95) and 23.7 kU_A/L sIgE to natural Pru p 3 (nPru p 3) (ImmunoCAP™ f420)) was used in immunoblot analysis for the detection of Pis s 3 in pea extract. In multipeptide microarray analysis, different combinations of negative controls were used. Table 5 shows the respective negative controls used in the individual microarray experiments. Serum N and PEI177 were from the internal serum collection of the Paul-Ehrlich-Institut, and DLab sera were bought from DLab Diagnose GmbH (Hamburg, Germany). These non-allergic subjects showed no sensitization to the respective investigated legume allergen. Sera A-J were provided by the Charité Berlin. Sera A-E were from atopic subjects (fx5 [egg white, milk, fish, wheat, peanut, soybean] negative; SX1 [*Dermatophagoides pteronyssinus*, cat dander, dog dander, timothy grass, cultivated rye, *Cladosporium herbarum*, common silver birch, mugwort] positive) and sera F-J from non-atopic subjects (fx5 negative; SX1 negative). PEI163, an in-house positive control serum, was additionally used in all immunoblot and microarray analyses. The measured sIgE levels (ImmunoCAP™) of PEI163 were (amongst others): > 100 kU_A/L sIgE to peanut extract, 38.5 kU_A/L sIgE to soybean extract and 16.73 kU_A/L sIgE to pea extract.

Table 5: Multipeptide microarray experiments and used controls.

Proteins written with forward slash were analyzed on the same array.

Microarray experiment	Negative controls
Ara h 1	Serum N, DLab71S1
Ara h 2.01/Ara h 2.02	Serum N, DLab71S1, DLab72S1
Pis s 1	Serum A-J

PA1/PA2	Serum N, PEI177
Gly m 5.03	Serum N
Gly m 8	Serum N

3.1.7 Plasmids

For the expression of recombinant proteins in *Pichia pastoris*, the yeast expression vector pPICZαA (Invitrogen/Thermo Fisher Scientific) was used. pPICZαA contains the Zeocin™ resistance gene allowing the selection of transformants. For the expression of rPis s 1 in *Escherichia coli*, the *E. coli* expression vector pET-11a (Novagen, Darmstadt, Germany) was used. This vector carries the gene for ampicillin resistance. Vector maps and multiple cloning sites are shown in the appendix (Figure A1 and Figure A2).

3.1.8 Primer

Table 6: Oligonucleotides used for colony PCR and DNA sequencing.

Primer	**Sequence**
5'AOX (forward)	5'-GACTGGTTCCAATTGACAAGC-3'
3'AOX (reverse)	5'-GCAAATGGCATTCTGACATCC-3'
Pis s 1 mid (forward)	5'-AGATCTGCGTGTTCTGGATCTG-3'
T7 promoter (forward)	5'-TAATACGACTCACTATAGGG-3'
T7 terminator (reverse)	5'-GCTAGTTATTGCTCAGCGG-3'

3.1.9 *Pichia pastoris* and *Escherichia coli* strains

Table 7: *Pichia pastoris* and *Escherichia coli* cloning and expression strains.

Strain	**Company**	**Genotype**	**Phenotype (*Pichia pastoris* only)**
X-33	Invitrogen/Thermo Fisher Scientific	Wild-type	Mut$^+$
SMD1168H	Invitrogen/Thermo Fisher Scientific	pep4	Mut$^+$ His$^+$
One Shot® BL21 (DE3)	Invitrogen/Thermo Fisher Scientific	F$^-$ *ompT hsdS*$_B$ ($r_B^- m_B^-$) *gal dcm* (DE3)	
Stellar™	Clontech Laboratories, Inc., Mountain View, CA, USA	*F-, endA1, supE44, thi-1, recA1, relA1, gyrA96, phoA, Φ80d lacZΔ M15, Δ(lacZYA-argF) U169, Δ(mrr-hsdRMS-mcrBC), ΔmcrA, λ–*	
One Shot® TOP10	Invitrogen/Thermo Fisher Scientific	F$^-$ mcrA Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G	

3.2 Methods

3.2.1 Cloning of DNA fragments into expression vectors

3.2.1.1 Restriction

For cloning of cDNA into pPICZαA or pET-11a, both expression vectors had to be linearized first. *P. pastoris* expression vector pPICZαA was linearized using FastDigest EcoRI restriction enzyme (Thermo Fisher Scientific) according to the manufacturer's instructions. For efficient linearization, the incubation time was extended to 1 h. For linearization of *E. coli* expression vector pET-11a, 1.5 μl FastDigest restriction enzyme NdeI (Thermo Fisher Scientific) was used per 1 μg plasmid DNA. The incubation time was 7 h at 37°C.

3.2.1.2 Agarose gel electrophoresis

Linearization of expression vectors and colony PCR reactions (described in 3.2.2.4) were analyzed on 1.5% agarose-gels in TAE buffer. DNA samples were loaded with 6x Orange DNA Loading Dye (Thermo Fisher Scientific) and separated together with GeneRuler™ 1 kb Plus DNA Ladder (ready-to-use; Thermo Fisher Scientific) for 10-20 minutes at 210-275 V. After electrophoresis, gels were stained using ethidium bromide for 30 minutes.

3.2.1.3 Purification of linearized vectors

Linearized vector DNA was subsequently purified using QIAquick® PCR purification KIT (QIAgen®). The procedure was according to the manufacturer's instructions. For elution of DNA, 30 μl H_2O was used. After purification, the DNA concentration was determined photometrically.

3.2.1.4 Cloning of DNA fragments into linearized pPICZαA or pET-11a

The codon optimized cDNA sequences encoding the investigated proteins without signal peptide were purchased from Geneart (Geneart Strings DNA Fragments, Thermo Fisher Scientific) and inserted into EcoRI-linearized purified pPICZαA or NdeI-linearized purified pET-11a. The cDNA sequences of all proteins listed in Table 8A were cloned into pPICZαA and the sequence of rPis s 1 was additionally cloned into pET-11a (Table 8B). Cloning into EcoRI restriction site of pPICZαA resulted in two additional amino acid residues (Glu-Phe) at the N-terminus of each protein. Whereas cloning into ATG cloning site NdeI in pET-11a resulted in an additional Met at the N-terminus of rPis s 1. In addition, in order to prevent N-glycosylation in *Pichia pastoris* potential N-glycosylation sites were predicted using NetNGlyc 1.0 Server (http://www.cbs.dtu.dk/services/NetNGlyc/) and substituted by an aspartic acid (D). Potential

N-glycosylation sites were found in Ara h 1, Pis s 1, PA2, Gly m 5.03 and Gly m 8. Further modifications of the recombinant proteins were linker and His_6-tag attached to the C-terminus of each protein expressed in *P. pastoris* (see e.g. Figure A3 in the appendix and Table 8A) and thrombin cleavage site, linker, and His_6-tag attached to the C-terminus of rPis s 1 expressed in *E. coli* (Figure A7 in the appendix and Table 8B). Modifications and accession numbers of the proteins are summarized in Table 8. In addition, Table 8 shows in the first column in parentheses the specific IUIS database nomenclature of the investigated allergen or as in the case of PA1 and PA2, the specific UniProt protein name. For simplification, abbreviated protein names (Table 8, bold) will be used in this thesis.

Sequences of DNA fragments used for cloning into pPICZαA or pET-11a and derived amino acid sequences are listed in the appendix, in Figure A3-A11.

Table 8: Accession numbers and modifications of recombinant proteins.

A

Recombinant protein (specific IUIS or UniProt name)	**UniProt accession number**	**Modifications in recombinant proteins**
rAra h 1, clone P41B (Ara h 1.0101)	P43238-1	N496D, linker*, His_6-tag
rAra h 2.01 (Ara h 2.0101)	Q6PSU2-2 variant G40E E130D	Linker*, His_6-tag
rAra h 2.02 (Ara h 2.0201)	Q6PSU2-1	Linker*, His_6-tag
rPis s 1 (Pis s 1.0101)	Q702P1-1	N345D, linker*, His_6-tag
rPA1 (PA1 B)	P62927-1	Linker*, His_6-tag
rPA2 (PA2a)	P08688-1	N56D, linker*, His_6-tag
rGly m 5.03 (Gly m 5.0302)	P25974-1 variant V28G	N328D, linker*, His_6-tag
rGly m 8 (Gly m 8.0101)	P19594-1	N99D, linker*, His_6-tag

B

Recombinant protein (specific IUIS or UniProt name)	**UniProt accession number**	**Modifications in recombinant proteins**
rPis s 1 (Pis s 1.0101)	Q702P1-1	Thrombin cleavage site, linker#, His_6-tag

(A) Recombinant proteins aimed to be expressed in *P. pastoris*. (B) rPis s 1 expressed in *E. coli*. Amino acid substitutions of variants and substitutions due to prevention of N-glycosylation refer to the UniProt protein sequence without signal peptide; *linker: VD; #linker: SSG.

Cloning into expression vectors was done using the In-Fusion® HD EcoDry™ Cloning Kit (Clontech Laboratories, Inc., Mountain View, CA, USA). For In-Fusion Cloning, the cDNA sequences of the recombinant proteins contained at each end additional bases homologous to the ends of the linearized vector in which the cDNA should be cloned (see appendix Figure A3-A11). In-Fusion® Cloning was done according to the manufacturer's instructions. Briefly, 100 ng of purified linearized vector was mixed with 100 ng of the DNA fragment and incubated according to the manufacturer's instructions. For transformation of Stellar™ cells, 30 µl competent cells and 3 µl of the In-Fusion® reaction mixture was used. Transformation reactions were spread on LB agar-plates containing either zeocin (75 µg/ml) or ampicillin (50 µg/ml).

3.2.1.5 Plasmid isolation and quantification of DNA

Plasmid DNA was isolated from 5 ml *E. coli* overnight cultures (37°C, 210 rpm) in LB medium with 75 µg/ml zeocin or 50 µg/ml ampicillin. Isolation was done using the QIAprep® Spin Miniprep Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. For elution of DNA, 40 µl H_2O was used.

Afterwards, DNA concentration was determined by measuring the absorbance at wavelength 260 nm and 280 nm using NanoPhotometer®.

3.2.1.6 Sequencing

Successful cloning of DNA fragments into pPICZαA or pET-11a was confirmed by Sanger sequencing (Eurofins Genomics GmbH, Ebersberg, Germany). For sequencing of pPICZαA constructs, primer 3'AOX with or without primer 5'AOX was used. pET-11a-Pis s 1 was sequenced and confirmed using primers T7, T7 term and Pis s 1 mid.

3.2.1.7 Transformation of plasmid DNA into *E. coli*

For amplification, plasmid DNA was transformed into One Shot® TOP10 chemically competent cells according to the manufacturer's instructions (Thermo Fisher Scientific, Document Part Number C4040). For transformation, 10 µl cells were incubated with 0,5 µl plasmid DNA for 30 min on ice. After a heat shock at 42°C for 30 sec, cells were incubated on ice for 2 min. 250 µl SOC medium were added and cells were shaked at 37°C for 1 h at 500 rpm. Afterwards, the transformation mix was plated on LB agar-plates containing either zeocin (75 µg/ml) or ampicillin (50 µg/ml). Of grown colonies plasmid DNA was isolated as described in chapter 3.2.1.5.

3.2.2 Expression of recombinant proteins using *Pichia pastoris*

3.2.2.1 Linearization and purification of pPICZαA constructs

For integration into the *Pichia* genome, pPICZαA plasmids containing the genes of interest had to be linearized. Linearization was done using the restriction enzyme SacI (FastDigest; Thermo Fisher Scientific). As the sequence of both Ara h 2 isoforms contained the SacI restriction site, the restriction enzyme BstXI (FastDigest; Thermo Fisher Scientific) was used for linearization. 10 μg of each plasmid was linearized using up to 10 μl restriction enzyme and an incubation time of 4 h at 37°C. Successful linearization was confirmed by agarose gel electrophoresis (see chapter 3.2.1.2). Restriction enzymes SacI and BstXI were inactivated by heating for 5 min at 65°C and 80°C, respectively. Prior to transformation of *Pichia pastoris*, linearized plasmids were purified using QIAquick® PCR purification KIT as described in chapter 3.2.1.3.

3.2.2.2 Preparation of electrocompetent *P. pastoris* X-33 cells

Competent cells were prepared according to the instructions in the EasySelect™ *Pichia* Expression Kit manual (Cat. no. K1740-01). *Pichia pastoris* X-33 cells were grown overnight in 5 ml YPD at 30°C and 200 rpm. 500 μl of this overnight culture was used to inoculate 500 ml fresh YPD medium in a 2 L flask. Culture was shaken overnight until an OD_{600} of 1.3-1.5 was reached. Cells were harvested by centrifugation at 1,500 x g for 5 min at 4°C. Afterwards, cell pellet was resuspended with 500 ml ice-cold, sterile H_2O. Cells were again harvested by centrifugation as described above and pellet resuspended with 250 ml ice-cold sterile H_2O. After a third centrifugation step, pellet was resuspended with 20 ml ice-cold 1 M sorbitol. This step was followed by a centrifugation step and a resuspendation step with 1 ml ice-cold 1 M sorbitol and by three wash steps composed of centrifugation and resuspendation with 500 μl ice-cold 1 M sorbitol. Finally, cells were harvested and the cell pellet was resuspended in ice-cold 1 M sorbitol to a final volume of ~1.5 ml. Cells were kept on ice before electroporation.

3.2.2.3 Transformation

80 μl of competent X-33 cells were mixed with 5 μg of the linearized purified pPICZαA constructs in an ice-cold electroporation cuvette (0.2 cm gap width) and incubated on ice for 5 min. Cells were pulsed at 1.5 kV, 25 μF and 200Ω. Immediately afterwards, 1 ml ice cold 1 M sorbitol was added and the contents were transferred to a 15 ml sterile tube. After 1-2 h incubation at 30°C different volumes were spread on YPDS plates containing 100 μg/ml Zeocin™. Until colonies grew, plates were incubated at 30°C.

3.2.2.4 Colony PCR

To confirm a successful integration of the construct/gene of interest into the *Pichia pastoris* genome, a PCR analysis was performed. Using 5'AOX and 3'AOX as primers, the PCR gives additional information about the Mut phenotype. Analyzing Mut^+ or Mut^S integrants resulted in two or one PCR product, respectively. One PCR product corresponds to the gene of interest and if present, the second to the AOX1 gene.

For PCR analysis, a single colony was picked and transferred to a 0.5 ml sterile tube. The DNA was released by a heating step for 1.5 min at 600 watt in the microwave and was afterwards resuspended in 20 μl sterile H_2O. The reaction setup is listed in Table 9.

Table 9: Reaction setup colony PCR.

Component	Amount
10X ThermoPol buffer (New England Biolabs)	1.8 μl
100 mM dNTPs (25 mM each)	0.26 μl
5'AOX Primer (100 pmol/μl)	0.54 μl
3'AOX Primer (100 pmol/μl)	0.54 μl
Taq Polymerase 5,000 units/ml (New England Biolabs)	0.18 μl
Pichia pastoris solution	2 μl
Sterile water	to 18 μl

After pipetting the PCR sample, PCR was performed according to the conditions listed in Table 10. Afterwards, PCR products were analyzed on a 1.5% agarose gel as described in chapter 3.2.1.2.

Table 10: PCR conditions.

Temperature	Time	Cycles
95°C	5 min	1 cycle
95°C	30 sec	30 cycles
50°C	45 sec	
68°C	1 min/kb	
68°C	5 min	1 cycle

3.2.2.5 Expression

All recombinant proteins were expressed using Mut^+ *Pichia* recombinant clones, except rPA2, which was expressed using a Mut^S recombinant clone. As the screening of *Pichia* clones by

colony PCR revealed that only clones of the Mut^S phenotype contained the gene of interest, i.e. PA2.

Protein expression was performed according to the manufacturer's instructions written in EasySelect™ *Pichia* Expression Kit manual with minor modifications. Briefly, a small-scale expression was used to determine optimal conditions for protein expression. Afterwards, expression was scaled up using up to 3 L of culture volume.

For the expression using Mut$^+$ *Pichia* recombinant clones the following protocol was used. A single colony was grown in 5 ml of BMGY medium overnight at 30°C and 210 rpm. The following day, this overnight culture was used to inoculate 650 ml of BMGY medium in a 5 L baffled flask. Culture was grown at 30°C and 210 rpm until it reached an OD_{600} of 2-6. Cells were harvested by centrifugation at 1,500 x g for 5 min at room temperature. For inducing protein expression, the cell pellet was resuspended to an OD_{600} of 1 in 3L BMMY medium. 750 ml aliquots were transferred to 5 L baffled flasks and incubated at 30°C at 210 rpm for 4 to 8 days. Incubation time was dependent on the respective recombinant protein. To maintain induction, 100% methanol was added to a final concentration of 0.5% every day. Finally, expression was terminated by centrifuging the culture twice at 4,000 x g for 15 min at room temperature. Supernatant was filtered (0.2 μm, asymmetrical polyethersulfone, Thermo Fisher Scientific) and stored until use at 4°C.

For the expression of rPA2 using a Mut^S *Pichia* recombinant clone the following protocol was used. A single colony was grown in 10 ml of BMGY medium at 30°C until an OD_{600} of 2-6 was reached. Afterwards, this culture was used to inoculate 1 L of BMGY medium. The culture was grown until again an OD_{600} of 2-6 was reached. Cells were harvested by centrifugation as described above. For induction, the cell pellet was resuspended in 200 ml BMMY medium. Culture was incubated at 30°C and 210 rpm for 8 days. Maintenance of induction and termination of expression was carried out as described for Mut$^+$ recombinant clones. Supernatant was filtered and stored until use at 4°C.

Of note, a recombinant X-33 clone carrying pPICZαA without insert/gene of interest was additionally used in every expression experiment as control for background protein expression.

3.2.2.6 Optimization strategies

Several optimization strategies were applied within this thesis. In the case of rAra h 2.01 and rAra h 2.02 proteolysis was decreased by the daily addition of ~10 ml 7x protease inhibitor stock solution (cOmplete Tablets, Mini, EDTA-free, Roche Diagnostics GmbH, Mannheim, Germany) to the 3 L culture supernatant. This optimization strategy was also applied for rPis s 1, however as the extent of the degradation was higher 1.5 ml 7x protease inhibitor stock solution

was added per 100 ml culture supernatant. In addition, proteolysis of rPis s 1 was tried to reduce by the daily addition of PMSF and EDTA each to a final concentration of 1 mM and by the use of a *Pichia pastoris* SMD1168H strain lacking protease A activity. The protocol for transformation and expression was identical to the protocol described above for *Pichia pastoris* X-33 strain. Expression of rAra h 1 was checked using multiple recombinant *Pichia pastoris* clones. Furthermore, culture supernatant was concentrated using Vivaspin 20 centrifugal concentrator (Merck) to check for a potential low expression level.

3.2.3 Expression of recombinant Pis s 1 using *E. coli* BL21 (DE3)

3.2.3.1 Transformation

50 μl chemically competent One Shot® BL21 (DE3) cells were mixed with 1 μl plasmid DNA and incubated on ice for 30 min. The following steps equaled the protocol described in chapter 3.2.1.7.

3.2.3.2 Expression

A single colony of BL21 (DE3) transformants was grown over night in 100 ml LB medium containing 50 μg/ml ampicillin. This overnight culture was used to inoculate 4 L of fresh LB medium containing 50 μg/ml ampicillin to an OD_{600} of 0.1. The culture was grown at 37°C and 210 rpm and at an OD_{600} of ~0.6 protein expression was induced by the addition of IPTG to a final concentration of 1 mM. Induction was carried out for 3 h at 37°C and 210 rpm. Afterwards cells were harvested by centrifugation at 7,000 x g for 10 min at 4°C. Resulting cell pellet was washed twice with cold (4°C) sterile H_2O and stored at -20°C until use.

3.2.3.3 Protein extraction using BugBuster™ Protein Extraction Reagent

To examine whether rPis s 1 was expressed in the soluble fraction or in inclusion bodies, cell culture samples were analyzed using the BugBuster™ Protein Extraction Reagent (Merck). Therefore, 2 ml *E.coli* culture from chapter 3.2.3.2 was centrifuged (16,000 x g, 10 min and 4°C) to obtain the cell pellet. Cell pellet was resuspended in 300 μl BugBuster reagent. Afterwards, 0.5 μl Benzonase® Nuclease (250 U/μl, Merck) and 43 μl 7x protease inhibitor stock solution (cOmplete Tablets, Mini, EDTA-free) were added and the suspension was incubated for 20 min at room temperature on an orbital shaker. The following steps were done according to the manufacturer's protocol (User Protocol TB245 Rev. F 1108). Samples were analyzed by SDS-PAGE as described in chapter 3.2.8.2.

3.2.4 Purification of recombinant proteins

3.2.4.1 Protein extraction using cell disruptor

Compared to the recombinant proteins secreted into *P. pastoris* cell culture supernatant, rPis s 1 was purified from *E. coli* inclusion bodies. Therefore, prior to purification, rPis s 1 had to be extracted from the cell. Cells were lysed using a cell disruptor and a pressure of 1,900 bar. For this, the frozen cell pellet was thawed on ice and resuspended in 15 ml 20 mM sodium phosphate (pH 7.6) including one protease inhibitor cocktail tablet (cOmplete Tablets, Mini, EDTA-free) and a spatula tip of lysozyme (from chicken egg white, 100,000 U/mg, Sigma-Aldrich). The suspension was stirred for 20 min at room temperature, a spatula tip of DNAse I (3230.7 U/mg, AppliChem) was added and stirring was continued for further 20 min. Afterwards, cells were lysed using a cell disruptor with a pressure of 1,900 bar. The resulting lysate was centrifuged at 12,000 x g at 4°C for 20 min. Pellet was resuspended in 15 ml denaturing buffer and stirred for 3 h at room temperature. The lysate was sterile filtered (0.22 μm, polyethersulfone, Carl Roth) and used for further purification.

3.2.4.2 IMAC of recombinant proteins expressed in *P. pastoris*

Filtered *P. pastoris* culture supernatants from chapter 3.2.2.5 were incubated overnight each with 5 ml Ni-beads (Ni-NTA Superflow, Qiagen, Hilden, Germany) equilibrated in washing buffer. Beads were pelleted by centrifugation (1,000 x g, 1 min, 4°C) and transferred to a Bioline HR glass column (Knauer) which was afterwards connected to the HPLC system.

The purification procedure was composed of a washing step using washing buffer at a flow rate of 0.5 ml/min (2-5 column volumes). Afterwards, bound target protein was eluted using elution buffer 1 at a flow rate of 0.5 ml/min. Elution fractions were analyzed on SDS-PAGE as described in chapter 3.2.8.2. Fractions containing target protein were either pooled and dialyzed against dialysis buffer 1 or further purified by size exclusion chromatography. For dialysis and all following dialysis, D-Tube™ Dialyzers (MWCO 3.5 kDa, Merck) were used.

3.2.4.3 IMAC of recombinant Pis s 1 expressed in *E. coli* inclusion bodies

Lysate from chapter 3.2.4.1 was incubated overnight with 8 ml Ni-beads equilibrated in denaturing buffer. After a washing step with denaturing buffer at a flow rate of 0.5 ml/min and 15 column volumes, bound target protein was refolded by gradually lowering the urea concentration from 8 M urea in denaturing buffer down to 0 M urea in renaturing buffer. The linear gradient was performed at a flow rate of 0.2 ml/min and ~15 column volumes. Afterwards, rPis s 1 was eluted using elution buffer 2. Elution fractions were analyzed by SDS-PAGE. Fractions containing high amounts of rPis s 1 were pooled and dialyzed against dialysis

buffer 2. rPis s 1 was sterile filtered (0.22 μm, polyethersulfone, Carl Roth), aliquots were snap-frozen and stored at -80°C until use.

3.2.4.4 SEC of recombinant proteins

For size exclusion chromatography either single IMAC elution fractions containing target protein or pooled IMAC elution fractions that were concentrated (Amicon® Ultra Centrifugal filters, Merck) were used. Prior to injection into sample loop (1 ml), fractions were sterile filtered using Spin-X® Centrifuge Tube Filters (0.22 μm; cellulose acetate, Sigma-Aldrich).
Size exclusion chromatography was performed using a prepacked BioFox 40/1200 SEC column (Knauer) connected to the HPLC system. Recombinant proteins were purified using dialysis buffer 1 at a flow rate of 0.2 ml/min (2 column volumes). SEC fractions were analyzed by SDS-PAGE and fractions containing target protein were pooled and dialyzed against dialysis buffer 1. After dialysis, rPA1 was once again filtered using Spin-X® Centrifuge Tube Filters. Aliquots of recombinant proteins were snap-frozen and stored at -80°C until use.

3.2.5 Total legume protein extraction

Dried mature pea seeds of *Pisum sativum* (cultivar Regina) and yellow soybeans of *Glycine max* (Hensel Soja-Kost, W. Schoenenberger GmbH & Co. KG, Magstadt, Germany), respectively, were washed with dH_2O and dried overnight. Peanut kernels of *Arachis hypogaea* cultivar Giant USA were used for protein extraction. For total protein extraction, seeds and kernels were ground to powder under liquid nitrogen. 2 g of soybean and pea flour, respectively, were extracted in 20 ml PBS for 4 h at 4°C. For total peanut extraction, 4 g peanut flour was extracted in 20 ml PBS for 4 h at 4°C. After total protein extraction, extracts were centrifuged at 13,000 x g for 30 min at 4°C. The protein containing supernatants were filtered (pore size 0.22 μm, polyethersulfone, Carl Roth) and stored at -80°C until use.

3.2.6 Reduction and alkylation

Peanut extract, rAra h 1 and rAra h 2.02 were reduced and alkylated as described by Albrecht and co-workers in 2009 (Albrecht et al. 2009). Briefly, peanut extract (11 mg), rAra h 1 (0.7 mg) and rAra h 2.02 (0.5 mg), respectively, were denatured using 5.3 M urea and 0.13 M Tris-HCl. Afterwards, samples were reduced with 28 mM dithiothreitol and alkylated using 55.5 M iodacetamide. Obtained reduced and alkylated peanut extract and recombinant peanut proteins were dialyzed overnight at 4°C against PBS and dialysis buffer 1, respectively.

3.2.7 Peptide synthesis and spotting

3.2.7.1 Synthesis and preparation of overlapping Celluspot™ peptides

Overlapping Celluspot™ peptides (length: 15 amino acids; offset: 4 amino acids) covering the entire primary sequence of all legume proteins listed in Table 8 were synthesized and processed as described earlier (Kühne et al. 2015). Peptides are derived from the mature natural proteins, i.e. without signal peptide and without the modifications of the recombinant proteins (N/D substitutions, additional amino acids due to cloning, thrombin cleavage site, linker and His_6-tag). Furthermore, peptides of mature Gly m 5.0301 (UniProt accession number P25974-1 variant F13L V28G F174L) were also synthesized. In addition to the in-house synthesized peptides of Ara h 2.0101 and Ara h 2.0201 containing a proline in the DPYSPS motif, the respective peptides containing hydroxyproline residues were purchased from Intavis AG. One biotinylated control peptide (sequence by Intavis AG: Bio-A-A-N-W-S-H-P-Q-F-E-K-A-A) containing an N-terminal biotin (Biotin ≥ 99% (TLC), Sigma-Aldrich) was in-house synthesized and also purchased from Intavis AG.

Peptide synthesis and processing were almost identical to the protocol published by Kühne and co-workers (Kühne et al. 2015) with only minor modifications. Briefly, peptides were synthesized on modified (Fmoc-protected) cellulose discs (CelluSpot discs, Intavis AG) as solid support. The synthesis followed the Fmoc-protection chemistry meaning that all amino acids were protected at their amino terminus by Fmoc. In addition, the amino acid side chains were permanently protected during the synthesis by Pbf, Trt, OtBu, Boc or tBu. Amino acids used for peptide synthesis were purchased from Intavis AG. The synthesis was a cyclic procedure of deprotection using piperidine, washing, activation of amino acids using HOBt/DIC, coupling, capping of un-reacted free amino groups using CapMixture and washing. After peptide synthesis, side chain protection groups were removed using Side-chain cleavage solution. Next, cellulose discs with bound peptides were dissolved using cleavage solution. Dissolved peptide-cellulose conjugates were stored until use at -20°C.

3.2.7.2 Peptide spotting

Overlapping 15-mer peptides were spotted in quadruplicate onto coated microscope slides using a Slide Spotting Robot (Intavis AG) according to the manufacturer's instructions (Intavis AG) and according to Kühne *et al.* (Kühne et al. 2015). For coating of microscope slides adhesive foil (Lasertab® Markers, Brady Deutschland, Egelsbach, Germany) was used. Peptides representing Ara h 1, Pis s 1 and Gly m 8 were spotted individually on separate array slides. Peptides representing Ara h 2.01 and Ara h 2.02 including peptides with hydroxyproline(s) were spotted onto the same array slide, with peptides shared by both isoforms being spotted

once for both isoforms. The same procedure was applied for peptides of Gly m 5.0301 and Gly m 5.0302. Due to no significant sequence similarity between PA1 and PA2, peptides representing both pea proteins were spotted onto the same array. The biotinylated control peptide was additionally spotted in different dilutions (1:20 and 1:80 in DMSO) on every array and served both as a position marker to simplify the grid alignment during array analysis, and as a quality marker to verify consistency of chemiluminescent detection. In addition, blank spots composed of peptide buffer (DMSO) without peptide were spotted as technical replicates on every array and served for the measurement of background signal. For Ara h 1, Ara h 2, Pis s 1, PA1/2, Gly m 5.03 and Gly m 8 arrays, 76, 266, 134, 210, 152 and 306 blank spots were spotted in duplicate, respectively. Per spot 0.04 μl peptide, control peptide or blank was spotted. Coomassie-staining of a randomly chosen subset of array slides confirmed successful spotting of all peptide spots.

3.2.8 Characterization of purified proteins, total protein extracts and a 27-mer Ara h 2.02 peptide

3.2.8.1 Measuring protein concentration

The concentration of recombinant proteins was determined either by measuring the absorbance at 280 nm using NanoPhotometer® or by using BCA™ Protein Assay Kit (Thermo Fisher Scientific). Usage of BCA™ Protein Assay Kit occurred according to the manufacturer's instructions. For nanophotometric analysis, proteins' extinction coefficients and molecular weights were computed using ExPASy ProtParam tool (http://web.expasy.org/protparam/).
For determining the protein concentration of protein extracts, Roti®-Nanoquant (Carl Roth GmbH) was used according to the manufacturer's instructions.

3.2.8.2 SDS-PAGE

Recombinant proteins and legume extracts were separated under reducing conditions using discontinuous SDS-PAGE according to the principle described by Laemmli (Laemmli 1970). For this, commercial NuPAGE™ 4-12% Bis-Tris SDS-PAGE precast gels (Invitrogen/Thermo Fisher Scientific) or self-prepared gels without polyacrylamide gradient (15% separating gel and 5% stacking gel) were used. The composition of the separating and stacking gels is shown in Table 2. The respective reagents were pipetted and mixed in their listed order (see Table 2).
Protein and extract samples were denatured in SDS-PAGE sample buffer (final concentration 1x) at 95°C for 5 min. Samples were loaded onto the gel and electrophoresis was carried out at a current of 25 mA in NuPAGE® MOPS SDS running buffer or in 1x SDS-PAGE running buffer. For molecular weight determination, low-molecular weight marker (GE Healthcare, Freiburg,

Germany) mixed with SDS-PAGE sample buffer or Spectra™ Multicolor Broad Range Protein Ladder (Thermo Fisher Scientific) were additionally loaded onto the gel.

3.2.8.3 Native PAGE

Soybean extract was further analyzed using native non-denaturing PAGE. For this purpose, the extract sample and low-molecular weight marker were each mixed with Native PAGE sample buffer (final concentration 1x) and loaded directly onto the gel (15% separating gel and 5% stacking gel). To prepare separating and stacking gel, the respective reagents listed in Table 2 were used in their listed order. Electrophoresis was carried out at a current of 25 mA in 1x Native PAGE running buffer.

3.2.8.4 Coomassie staining

After electrophoresis, gels were washed in dH_2O for several minutes and subsequently stained in Coomassie staining solution for 5 min. For destaining, gels were incubated in Coomassie destaining solution until unbound Coomassie was fully removed and the background of the gel reached clarity. After destaining, gels were washed in dH_2O and scanned for documentation.

3.2.8.5 Immunoblot

Recombinant proteins (1 μg/cm) and legume extracts (20 μg/cm) were loaded onto NuPAGE™ 4-12% Bis-Tris SDS-PAGE precast gels and separated under reducing conditions as described in chapter 3.2.8.2. In addition, soybean extract (20 μg/cm) was separated under native non-denaturing conditions as described in chapter 3.2.8.3.

In order to analyze rAra h 1 expression in *Pichia pastoris*, an aliquot of cell culture supernatant was loaded onto a gel without polyacrylamide gradient (15% separating gel and 5% stacking gel) and separated as described above. As positive controls in this experiment, 2.5 μg/cm rBet v 1 (containing a His-tag) and 15 μg/cm peanut extract were simultaneously analyzed in separate lanes of the same gel.

After electrophoresis, samples were semi-dry blotted onto nitrocellulose membranes (0.2 μm, GE Healthcare). Therefore, the membrane was equilibrated in anode buffer 2 for 30 min. For protein transfer, 3 and 2 blotting papers (grade GB003, Whatman, Sigma-Aldrich) dipped into anode buffer 1 and anode buffer 2, respectively, were laid on top of each other on a semi-dry blotter. On top of the blotting papers dipped in anode buffer 2, the equilibrated nitrocellulose membrane and the gel dipped into anode buffer 2 were laid. Finally, 3 blotting papers moistened with cathode buffer were laid on top. Blotting was carried out for 1 h at 0.8 mA/cm^2. After blotting, proteins on nitrocellulose membranes were stained with Ponceau S. After

scanning for documentation, membranes were cut into 2 mm strips and destained using TBS. Strips were blocked with blocking buffer for 2 x 30 min (one exception: stripes of blotted cell culture supernatant of *Pichia pastoris* were blocked using TBS 0.3% Tween for 2 h). After blocking, strips were washed with washing buffer for 5 min and incubated over night with 10 μl of patients' sera. As negative control, serum from a non-allergic subject (N) was used. All sera were 1:60 diluted in incubation buffer (10 μl serum + 590 μl incubation buffer) and sera from peanut and soybean patients were, prior to incubation on blot strips, defatted by centrifugation at 12,000 rpm at 4°C for 10 min (repeated twice). For the analysis of *P. pastoris* cell culture supernatant, the primary antibodies mouse anti-$(His)_6$-tag and mouse anti-Ara h 1 (PN-t) were diluted 1:750 in TBS 0.05% Tween 0.1% BSA. The next day, strips were washed with washing buffer for 5 x 5 min, followed by incubation of secondary antibody mouse anti-human IgE-HRP (diluted 1:10,000 in incubation buffer) for 1 h. For the detection of bound mouse antibodies (anti-$(His)_6$-tag and anti-Ara h 1), goat anti-mouse IgG-HRP diluted 1:20,000 in TBS (0.05% Tween, 0.1% BSA) was incubated on the strips for 1 h. After incubation of secondary antibody, strips were washed 5 times with washing buffer for each 5 min. Bound IgE and bound IgG antibodies were detected using LumiGLO Reserve™ Chemiluminescent Substrate Kit according to manufacturer's instructions (KPL, Gaithersburg MD, USA). Chemiluminescence was recorded using Imager system Fusion FX (firmware version 1.0.12, Vilber Lourmat Deutschland GmbH).

3.2.8.6 IgE immunoblot inhibition analysis

For IgE immunoblot inhibition, pea extract was separated and blotted as described above. Patients' sera were preincubated for 2 h at room temperature with either 50 μg rPis s 1, rPA1 or nPru p 3 (in PBS) prior to overnight incubation on blot strips. In addition, patients' sera were preincubated with the respective protein buffer without inhibitor (serum uninhibited). As negative control, serum N preincubated with the respective protein buffer without inhibitor was used. Subsequent steps of the immunodetection were identical to chapter 3.2.8.5.

Using Fusion-Capt Advance FX7 16.02 Software (Vilber Lourmat Deutschland GmbH) densitometric quantification of the IgE inhibition by rPis s 1 was carried out. Background signal intensity expressed as signal intensity of the non-allergic serum N was subtracted from each pea patient analyzed on the same membrane (N1: pea patients' sera 1-11; N2: pea patients' sera 12-19) and subsequently percent inhibition was calculated using to the following equation:

$$100 - \frac{\text{Signal intensity of serum inhibited}}{\text{Signal intensity of serum uninhibited}} \text{ x } 100$$

3.2.8.7 Densitometric quantification of sIgE levels

Specific IgE levels to recombinant proteins were densitometrically quantified using Fusion-Capt Advance FX7 16.02 Software (Vilber Lourmat Deutschland GmbH).

For the quantification of sIgE to rAra h 1, rAra h 2.01, rAra h 2.02 and rGly m 5.03 the corresponding ImmunoCAP™ values of selected sera were available for each calibration. Calibration in the quantification of rAra h 1 sIgE levels was done against 1 μg/cm rAra h 1 and a 3 fold serial dilution (1:9 to 1:729) of serum RS14239 having 103.61 kU_A/L sIgE to rAra h 1. The quantification of rAra h 2.01 and rAra h 2.02 sIgE levels was performed using a calibration done against 1 μg/cm rAra h 2.02 and serum RS14231 (54.1 kU_A/L sIgE to Ara h 2). For calibration, serum RS14231 was used undiluted, diluted 1:2, 1:5, 1:20 and 1:50 in incubation buffer. In the case of the quantification of rAra h 2.01 sIgE, one further dilution (1:150) was used for calibration. rGly m 5.03 sIgE was quantified using undiluted PEI163 (27.32 kU_A/L Gly m 5 sIgE) and 1 μg/cm rGly m 5.03 for calibration. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated in these analyses by multiplying the specific IgE concentration (kU_A/L) of the non-allergic control serum N analyzed on the same immunoblot membrane by two and four, respectively.

As for rPis s 1 such ImmunoCAP™ values were not available due to the lack of commercially available Pis s 1 CAPs, a different strategy for the quantification of rPis s 1 specific IgE was developed.

rPis s 1 sIgE was quantified after linear regression of sIgE level (kU_A/L) against densitometric signal intensity (volume). Calibration of sIgE levels was based on the densitometric volume values of sera from pea patients 3, 11, and 14 (Figure 21A), assuming 32.10 kU_A/L, 23.10 kU_A/L, and 6.5 kU_A/L rPis s 1 sIgE, because rPis s 1 fully inhibited 32.10 kU_A/L (96.55% inhibition), 23.10 kU_A/L (98.17% inhibition), and 6.5 kU_A/L (99.93% inhibition) pea total protein specific IgE in immunoblot inhibition (Figure 22, Table 26). Accordingly, sIgE to pea total protein was merely constituted by rPis s 1 sIgE in these sera and is therefore identical to it. Finally, rPis s 1 sIgE of sera from all pea patients was calculated from the individual densitometric volume value using the following linear regression equation: y(sIgE)= 7E-07x(volume) + 3.8242; R^2=0.9976). In this analysis, the LOD and the LOQ were defined as two times and four times respectively the volume value of the non-allergic control serum N resolved for rPis s 1 sIgE.

Table 11 summarizes the respective allergens with the corresponding patient sera that were analyzed on the same membrane as non-allergic control serum N1 or N2.

Table 11: Control sera used for calculating limit of detection in immunoblot analyses.

Allergen	Control serum	Patients
rAra h 1	Serum N1	Peanut patients 24-34
rAra h 1	Serum N2	Peanut patients 1-23 and 35
rAra h 2.01	Serum N1	Peanut patients 1-5 and 24-35
rAra h 2.01	Serum N2	Peanut patients 6-23
rAra h 2.02	Serum N1	Peanut patients 1-5 and 24-35
rAra h 2.02	Serum N2	Peanut patients 6-23
rGly m 5.03	Serum N	Soybean patients 1-21
rPis s 1	Serum N1	Pea patients 1-7
rPis s 1	Serum N2	Pea patients 8-19

3.2.8.8 Measurement of Ara h 2.01 sIgE levels using ImmunoCAP™

Using Phadia 100 instrument from Thermo Fisher Scientific sIgE levels to rAra h 2.01 (ImmunoCAP™ component f423) of the peanut study population were additionally quantified. Calibration was done in a range from 0.001 to 100 kU_A/L with intermediate steps at 0.35 kU_A/L, 0.70 kU_A/L, 3.5 kU_A/L and 17.5 kU_A/L. Handling of Phadia 100 instrument and measurement of rAra h 2.01 sIgE levels were done by Elke Haberkorn (Paul-Ehrlich-Institut).

3.2.8.9 Mediator release assay

The biological activity of legume extracts, recombinant proteins and two 27-mer synthetic peptides of Ara h 2.02 was assessed using a mediator release assay that is based on immortal rat basophilic leukemia cells that can be passively sensitized with human IgE. Two 27-mer peptides (D-P-Y-S-P-S-Q-D-P-Y-S-P-S-Q-D-P-D-R-R-D-P-Y-S-P-S-P-Y), one having hydroxylated proline residues instead of proline residues (underlined positions) were purchased from PT Peptide Technologies GmbH (Berlin, Germany). Both 27-mer peptides were ordered with a purity of > 90%. Quality and identity of both purchased peptides was confirmed by HPLC and MS analysis according to the commercial provider.

The mediator release assay followed the protocol published by Vogel *et al.* (Vogel et al. 2005). Briefly, rat basophilic leukemia (RBL-2H3) cells expressing the α-chain of the high-affinity IgE receptor (FcεRI) were sensitized overnight with different serum pools that were diluted 1:10 in RBL medium. In the peanut project, the following serum pools were generated for mediator release assay: serum pool 1 (peanut patients 3 and 17), serum pool 2 (patients 6-8, 10, 12, 15, 18 and 21-23) and serum pool 3 (peanut patients 2, 4, 5, 13 and 19). Sera for serum pools were selected according to the results of immunoblot and multi-peptide microarray analyses.

As serum agglutination was observed during mediator release pretesting, peanut serum pools were pretreated with thrombin (Sigma-Aldrich; 250 U). Therefore, each peanut serum pool was

incubated with a final concentration of 1 U/ml thrombin (dissolved in 1M $CaCl_2$). After 1 h at room temperature, samples were centrifuged (4°C; 16,000 x g, 20 min) and supernatants were used for overnight sensitization of RBL-2H3 cells.

In addition, in the pea project one serum pool composed of pea patients 1, 3, 4, 8 and 11 was generated (here: no thrombin addition was necessary). After overnight sensitization, RBL-2H3 cells were washed and afterwards stimulated for degranulation with different amounts of either legume extract, recombinant proteins or 27-mer peptides. As negative control in the pea project, cells were additionally stimulated with BSA. In the peanut project, no BSA was used as negative control in mediator release assay due to an observed serum IgE binding of peanut-allergic patients (pool 2) to BSA in immunoblot analysis (details not shown). Therefore, serum N served as negative control in peanut mediator release assay meaning that RBL-2H3 cells were additionally sensitized with serum N (1:20) and afterwards stimulated with all tested molecules.

Total mediator release was determined by cell lysis using 1% Triton X-100 (Sigma-Aldrich) and spontaneous release by incubating sensitized cells in buffer without allergen. Released β-hexosaminidase induced by either legume extracts, recombinant proteins or peptides was measured photometrically. Specific mediator release was expressed as percent of total mediator release after subtracting spontaneous release.

3.2.8.10 Circular dichroism spectroscopy

CD spectra of recombinant proteins and 27-mer peptides were recorded using a CD spectropolarimeter. Proteins and peptides were measured at 20°C in dialysis buffer 1 (exception: rPis s 1 measured in 20 mM sodium phosphate, 100 mM NaCl and 0.2 mM EDTA pH 7.6) in a quartz cuvette with a path length of 0.1 cm. Measurement settings were: band width 1 nm, step width 1 nm, scanning speed 50 nm/min, accumulation 10 (exception: rPA2: accumulation 5). Recombinant proteins and 27-mer peptides were measured at 2 to 11 μM and the respective buffer spectrum was subtracted. For calculating the mean molar ellipticity per residue, the following published equation was used (Pokoj et al. 2010):

$$[\theta_{MRW}] = 100 \times \frac{\theta \text{ [measured ellipticity in mdeg]}}{\text{N [number of amino acid residues] x c [protein concentration in mM] x d [path length in cm]}}$$

3.2.8.11 Dynamic light scattering (DLS)

Hydrodynamic radii of recombinant proteins were determined by DLS. Proteins were measured in UV cuvettes (10 mm; Carl Roth) in a Zetasizer instrument (software v6.12) in the same buffers used for CD spectroscopy. The individual protein concentrations ranged from 3 μM to 42 μM. Measurements were done at 25°C and were done in triplicate for each protein.

3.2.8.12 Mass spectrometry analysis

Natural proteins from crude legume extracts and recombinant proteins were separated using SDS-PAGE, stained with Coomassie and afterwards analyzed with liquid chromatography mass spectrometry (LC-MS^E). Mass spectrometry (MS) analyses were performed by Anna Engin, Luisa Schwaben, Dr. Jelena Spiric and Dr. Andreas Reuter at the Proteomics Core Facility of the Paul-Ehrlich-Institut and have not been part of my own experimental work. Nevertheless, the experimental methods are presented here for understanding and to allow the reader to assess the validity of the results and of the conclusions drawn. This follows a concept previously applied in the PhD thesis of Dr. Felix Husslik (PhD thesis 2016, Department of Chemistry, Technische Universität Darmstadt). In addition, all parameters used specifically for this study are given in detail. This applies especially to devices, databases and amino acid sequences.

“In-gel digestion:

The protein spots were excised and destained three times for 15 min in destaining solution. Reduction and alkylation of cysteine residues was carried for 15 minutes at 200 rpm (thermomixer, Eppendorf) with reduction and alkylation solution, respectively. The gel plugs were dehydrated with 100% acetonitrile, vacuum dried (Savant Speed Vac®, Thermo Fisher Scientific) and were rehydrated in 25 mM NH_4HCO_3 containing 75 ng/μl of trypsin (trypsin from porcine pancreas, Sigma-Aldrich). After initial digestion for 3 h at 37°C, elution buffer was added and further digestion was allowed over night at 37°C in a thermal cycler (Bibby Scientific, Staffordshire, UK). The digestion was stopped by adding of 5% formic acid to a volume of 10% of the total digestion mixture. The samples were stored at -80°C until further MS analyses.

LC-MS analyses:

Trypsin digested proteins were analyzed using the nanoACQUITY® UPLC online coupled with nano ESI interface to a Q-TOF MS (Synapt, Waters, Manchester, UK). The solvent system consisted of solvent A (water with 0.1% (v/v) formic acid), and solvent B (acetonitrile with 0.1% (v/v) formic acid). Peptides were initially trapped on-line at a flow rate of 5 μl/min using the trap column (nanoACQUITY Trap C18, 5 μm particle size, 180 μm × 20 mm). The peptide

mixture was separated using a reverse-phase analytical column (nanoACQUITY C18, 1.7 μm particle size, 100 μm × 100 mm; Waters) at a flow rate of 0.5 μl/min. The solvent composition was at 97% A for 1 min, followed by a linear gradient to 60% A for 30 min, and continued with 95% B for 1 min. The column was subsequently equilibrated at 97% A for 18 min. Glu-1-Fibrinopeptide at 1pmol/μl, delivered from the auxiliary pump of the nanoACQUITY® UPLC at a 0.5 μl/min flow rate to the reference sprayer of the interface was measured every 20 sec to serve as a lock mass during the entire sample run. The mass spectrometer was operated in data independent MS^E mode at a positive polarity and V mode (Silva et al., 2005; Silva et al., 2006). Scan rate was set to 0.4 sec. The collision energy was at a constant value of 4 V during low-energy scans and ramped from 15-30 V during high energy scans. Data were acquired from 50 to 1,990 m/z.

Data processing and database search:
Raw data files were processed using ProteinLynx Global Server (PLGS) version 2.4 (Waters). The processing parameters were set as follows: Retention time window, chromatographic peak width and MS TOF resolution were set to automatic, lock mass for charge state +1 and for charge state +2 were defined as 684.3469 Da and 785.8426 Da, respectively. Low energy threshold was set to 250.0 counts, elevated energy threshold to 100.0 counts and intensity threshold to 1,500 counts. The search parameters for protein identification were a maximum of one missed cleavage site, a minimum of three fragment ion matches per peptide, a minimum of seven fragment matches per protein and a minimum of one peptide match per protein with a set false positive rate of 4%. Carbamidomethylation of cysteine (C) was defined as a fixed modification. Variable modifications were restricted to deamination of asparagine (N) and glutamine (Q), and oxidation of methionine (M)."[1]

Compared to the cited procedure, two different instruments, Synapt G1 and Synapt G2si, were used in this PhD project (Table 12). In addition, regarding LC-MS analysis, peptide mixtures of nPA1 and nPA2 were separated using a different analytical column (ACQUITY UPLC M-Class Peptide HSS T3 Column 10K psi, 1.8 μm particle size, 75 μm X 150 mm). Moreover, raw data files were processed using ProteinLynx Global Server (PLGS) version 2.4 Synapt G1 data or PLGS 3.01 Synapt G2 data (Waters).
Finally, the identities of the proteins were confirmed using different UniProt-derived databases amended with the amino acid sequences of the recombinant proteins as outlined in Table 12.

[1]Husslik, Felix (2016). Analysis of the IgE epitope profile of the soybean allergen Gly m 4. PhD thesis, page 45-47, Technische Universität Darmstadt. URN: http://nbn-resolving.de/urn:nbn:de:tuda-tuprints-57360.

Table 12: Instruments, databases and sequences used for MS^E analyses.

Protein	Instrument	Database	Added sequence[#]	Amino acid sequence of recombinant protein
rAra h 1[*]	Synapt G1	UniProt restricted to reviewed entries of all species	-	-
rAra h 2.01	Synapt G1	UniProt restricted to reviewed entries of all species	PEI077	underlined sequence in Figure A4 in the appendix
rAra h 2.02	Synapt G1	UniProt restricted to reviewed entries of all species	PEI129	underlined sequence in Figure A5 in the appendix
natural proteins from peanut extract	Synapt G1	UniProt restricted to reviewed entries of all species	-	-
rPis s 1	Synapt G1	UniProt restricted to reviewed entries of eucaryotic organisms	PEI112	underlined sequence in Figure A7 in the appendix
rPA1	Synapt G1	UniProt restricted to reviewed entries of all species	PEI081	underlined sequence in Figure A8 in the appendix
rPA2	Synapt G1	UniProt restricted to reviewed entries of all species	PEI080	underlined sequence in Figure A9 in the appendix
nPA1	Synapt G2si	UniProt restricted to reviewed entries of all species	-	-
nPA2	Synapt G2si	UniProt restricted to reviewed entries of all species	-	-
rGly m 5.03	Synapt G1	UniProt restricted to reviewed entries of all species	PEI078	underlined sequence in Figure A10 in the appendix
rGly m 8	Synapt G1	UniProt restricted to reviewed entries of all species	PEI075	underlined sequence in Figure A11 in the appendix
natural proteins from soybean extract	Synapt G1	UniProt restricted to reviewed entries of all species	-	-

[*] provided by Dr. Jonas Lidholm (Thermo Fisher Scientific); [#] UniProt database was amended with the amino acid sequence of the recombinant protein.

3.2.8.13 Modeling of investigated proteins

For modeling of protein structures, SWISS-MODEL Workspace server available at https://swissmodel.expasy.org/ was used. For this purpose, full-length mature natural protein sequences were used. The protein structure of Ara h 1 was modelled using Ara h 1 pdb 3SMH (sequence identity 99.8%) as template. The structures of Ara h 2.01 and Ara h 2.02 were modelled using the structure of Ara h 6 (pdb 1W2Q) as template. Ara h 6 showed 66.1% and 64.4% sequence identity to Ara h 2.01 and Ara h 2.02, respectively. Using the structure of the

β-subunit of Gly m 5 (pdb 1UIJ) showing 59.4% sequence identity, the structure of Pis s 1 was modelled. After modeling, 3-D images were generated using PyMOL Molecular Graphics System (Version 1.5.0.4 or Version 2.0.6 Schrödinger, LLC, New York, USA).

3.2.9 Peptide analysis

3.2.9.1 IgE immunodetection

IgE-binding peptides were detected following the protocol published by Kühne *et al.* with some modifications (Kühne et al. 2015). Briefly, slides were blocked with blocking buffer for 2 h and afterwards incubated for 5 min in washing buffer. After drying the slides for at least 1 h at room temperature, 45 μl of defatted patient serum or control serum were applied to the array slide underneath a glass cover slip with spacer and incubated overnight. For defatting, serum samples were centrifuged twice at 12,000 rpm at 4°C for 10 min. The next day, the glass cover slip was removed and slides were washed 5 times with washing buffer. Afterwards, the slides were simultaneously incubated for 2 h with Streptavidin-HRP (SouthernBiotech, diluted 1:300,000) and mouse anti-human IgE-HRP in blocking buffer (diluted according to the investigated allergen). Ara h 1 and Ara h 2 slides were incubated with mouse anti-human IgE-HRP diluted 1:25,000 and 1:50,000, respectively. For the Pis s 1 and PA1/PA2 microarrays mouse anti-human IgE-HRP was diluted 1:5,000. Gly m 5 and Gly m 8 slides were incubated with mouse anti-human IgE-HRP diluted 1:10,000. Next, slides were washed 4 times with washing buffer and, after incubating the slides for 1 min with LumiGLO Reserve™ Chemiluminescent Substrate, signals were recorded on X-ray films.

3.2.9.2 IgE inhibition immunoassay

For IgE inhibition experiments, serum pools were preincubated with the respective inhibitors for 3.5-4 h at room temperature. Table 13 shows the investigated peptide microarray with the generated serum pools and the used inhibitors. Sera were pooled based on comparable IgE-binding pattern to the investigated peptides according to microarray analysis. As a reference, the respective serum pool was incubated with the respective protein buffer without inhibitor (dialysis buffer 1 or 2). Overnight incubation on array slides and all subsequent steps of the immunodetection were done as described above.

Table 13: Serum pools and inhibitors used for IgE inhibition microarray assay.

Peptide microarray	Serum pools	Inhibitor
Ara h 1	Pool 1: Peanut patients 10, 12, 18 and 21 Pool 2: Peanut patients 6, 15, 17 and 23	20.5 μg rAra h 1
Ara h 2	Peanut patients 6-8, 10, 12, 15, 18 and 21-23	13.5 μg rAra h 2.02 or 9.5 μg native peanut extract or 9.5 μg red/alk peanut extract
Pis s 1	Pool 1: Pea patients 1, 4 and 8 Pool 2: Pea patients 3, 10, 11 and 13	30 μg rPis s 1

3.2.9.3 Microarray data analysis

X-ray films were scanned at 600 dpi and saved as 16-bit grayscale TIF files. Using Adobe® Photoshop® (Version 13.0.1 x64), each slide was cropped to the size of a microscope slide (1796 x 615 pixel). Afterwards, grayscales were inverted using Image J 1.48v (Rasband, NIH, Bethesda, MD, USA; https://imagej.nih.gov/ij/) software and inverted images were again saved as TIF files.

Subsequently, TIGR Spotfinder 3.2.1 was used as software tool for analyzing the signal intensity of every spot on the array slide (Saeed et al. 2003). Here, in addition to IgE-bound peptides, biotinylated control peptides were used as position markers for grid alignment. TIGR Spotfinder program setting during spot analysis were: segmentation method circle, diameter 25, background correction off. After analyzing all spot signal intensities on the array, data were saved as mev-files and subsequently stored for further analysis as xlsx-files. For all following calculation steps, the median intensity value in channel A (MedA) was used for each spot. In the next step, the signal intensity of every peptide was transformed into a Z-score. Z-score transformation was performed as described by Hansen *et al.* with slight modifications (Hansen et al. 2016). Briefly, the median signal intensity (Si) of quadruplicate peptide signals was calculated for every peptide. Exceptions were sera of peanut patients 19 and 25 on Ara h 1 peptides and sera of pea patients 2, 8 and 10 on Pis s 1 peptides where only one segment (peanut patients 19 and 25: right segment; pea patients 2, 8 and 10: left segment) with duplicate peptide spots could be analyzed for each patient serum. Next, the constant mean (m) was calculated from all blank spots on the same array. The constant mean was obtained by a step by step calculation of the mean of the blanks excluding blank signal intensities outside mean ± 2 standard deviation. Once the constant mean was obtained, the standard deviation (s) was calculated of the blank spots representing the constant mean. Exception in the

calculation of the constant mean of the blanks was peanut patient 21 on Ara h 2 peptides, where not all blanks (2 x 266 vs. 2 x 138 blanks) were used for the calculation due to too high signal intensities of Ara h 2 peptides that overexposed too many neighbouring blank spots. Finally, Z-scores were calculated for every peptide according to the following equation:

$$\text{Z-score} = \frac{\text{Si - m}}{\text{s}}$$

In the last step, non-specific binding to peptides was removed by subtracting from the Z-score of each peptide the maximum Z-score of the same peptide probed with control serum. As controls for multipeptide microarray immunoassays, different sera and serum combinations were used which are summarized in Table 5. Important note: in every immunodetection, at least one control serum was included.

For defining positive serum IgE binding to an individual peptide, a Z-score of > 2 after control subtraction was used as criterion.

For calculating percent inhibition in IgE inhibition experiments, Z-scores of serum pools (uninhibited and inhibited) were calculated as described above except that no maximum Z-score of the controls was subtracted afterwards. Solely uninhibited peptides with a Z-score > 2 were used for further calculation of the percent inhibition. This step should ensure that, despite of no control subtraction, just uninhibited signals above the respective microarray background were used. In addition, inhibited peptides with a Z-score < 0 were set to zero. Finally, percent inhibition was calculated for every peptide according to this formula:

$$100 - \frac{\text{Z-score serum pool inhibited}}{\text{Z-score serum pool uninhibited}} \text{ x } 100$$

3.2.9.4 Selection of candidate diagnostic peptides

For each investigated allergen, candidate diagnostic peptides showing specific IgE binding only in the respective legume-allergic subjects were selected according to criteria established within this PhD thesis. At first, candidate peptides should be exclusively recognized by the allergic patients. Peptides recognized by tolerant patients were excluded from the selection. Secondly, candidate peptides should have a high signal intensity. Therefore, for every positive IgE-bound peptide (Z-score > 2 after control subtraction), the median Z-score was calculated of all allergic patients; peptides with Z-score ≤ 2 were excluded from the median calculation. Subsequently, peptides with a median Z-score of > 5 were selected for further analysis. Further, only peptides showing an IgE binding in relation to the full-length allergen were selected. Therefore, only

peptides were further selected which showed at least 30% inhibition of their IgE binding by the corresponding full-length protein in at least one inhibition experiment. Next, candidate diagnostic peptides should contain distinct amino acid sequences and represent distinct protein areas and therefore an offset of ≥ 4 peptides was applied between two peptides. If there were several options, the peptide with the higher IgE-binding frequency among the allergic patients was selected. If there were still several possibilities left, because multiple peptides may show the same IgE-binding frequency, the peptide with the highest median Z-score (calculated from positive IgE-bound peptides) was selected.

From all preselected peptides fulfilling the above-mentioned criteria those peptides that most frequently bound IgE (≥ median frequency of preselected peptides) were finally selected as candidate diagnostic peptides.

3.2.10 Receiver operating characteristic (ROC) curve analysis

Using SigmaPlot 13.0 (Systat Software GmbH, Erkrath, Germany) ROC curve analyses were performed. The following software settings were used for the analyses: data format indexed, data type paired, classification variable clinical phenotype/status (allergic or tolerant), positive state allergic, positive direction high. The following test variables were analyzed: sIgE levels to legume extracts, to rAra h 2.01 (both determined by ImmunoCAP™ analysis), to recombinant legume proteins (quantified by immunoblot analysis), and maximum Z-scores of selected candidate diagnostic peptides (determined by multipeptide microarray analysis) of legume-allergic and tolerant patients. Prior to ROC curve analysis, peanut extract and rAra h 2.01 sIgE levels > 100 kU_A/L were set to 100 kU_A/L as otherwise the software excluded these patients from analysis. A preliminary in silico analysis revealed that setting values > 100 kU_A/L to 100 kU_A/L or to randomly chosen 500 kU_A/L resulted in both cases in the same AUC (area under curve) and the same 95% confidence interval. In addition, prior to ROC curve analysis sIgE levels to recombinant peanut and soybean proteins that were quantified by immunoblot analysis were subtracted by two times the quantified sIgE level of the non-allergic control serum analyzed on the same membrane (N1 or N2). In the pea project, rPis s 1 sIgE levels were subtracted by two times the densitometric signal intensity of the non-allergic control serum N1 or N2 resolved for sIgE. Moreover, the maximum Z-score of the identified candidate diagnostic peptides was calculated for each individual patient, and used for ROC curve analysis. For maximum calculation, the Z-scores after control subtraction were used.

4 Results

4.1 Peanut project

4.1.1 Study population

In total, sera from 35 children were included in this peanut project (Table 14). The inclusion criterion was a sensitization to peanut extract, expressed by a peanut extract ImmunoCAP™ result of ≥ 0.35 kU_A/L. 23 of these children (patients 1-23) had symptomatic peanut allergy confirmed by double-blind placebo-controlled food challenge (16/23), single-blind controlled food challenge (1/23) or open food challenge (5/23). In patient 23 peanut allergy was confirmed by food challenge, however the exact type of challenge was unknown. Peanut allergy was excluded in the remaining 12 children (patients 24-35) by double-blind placebo-controlled food challenge (8/12) or open food challenge (4/12).

The allergic symptoms during food challenge ranged from grade II to grade V with V being the most severe grade. Severe symptoms (grade IV-V) were reported in 9/23 (39%) patients. In addition, no mild (grade I) symptoms were observed highlighting the high allergenicity of this legume in children. Due to a lack of symptom data, patient 18 was excluded from this calculation.

Total IgE ranged from 16.8 to 1985 kU_A/L in peanut-allergic children (median 296 kU_A/L) and from 10.3 to 1276 kU_A/L in peanut-tolerant children (median 514 kU_A/L) demonstrating a significant overlap in the total IgE level between both study groups. In contrast, peanut extract sIgE level was elevated in the peanut-allergic group compared to the tolerant group with median peanut sIgE of 24.5 kU_A/L and 1.93 kU_A/L, respectively. Additional characteristics are listed in the table below.

Serum N, DLab71S1 and DLab72S1 served as negative controls for immunoblot and microarray analyses.

Table 14: Clinical characteristics of the peanut study population.

Patients allergic to peanut (patients 1-23), patients sensitized to peanut without clinical symptoms (patients 24-35) and control sera (N, DLab71S1 and DLab72S1).

Patient No.	Age (y)*/sex	Clinical evidence	Symptoms to peanut	Severity grading#	Total IgE (kU_A/L)‡	Peanut sIgE (kU_A/L)‡
1	3/f	DBPCFC	V	II	n.d.	28.80
2	1/m	OFC	P, GU, AE	II	63.70	12.80
3	5/f	SBCFC	GU, W	IV	n.d.	23.90
4	1/f	DBPCFC	GU	II	16.80	4.22
5	3/m	DBPCFC	GU	II	n.d.	1.98
6	6/m	OFC	V, S, Cg	IV	451.00	>100

7	3/f	DBPCFC	W	IV	n.d.	14.10
8	4/f	DBPCFC	RA	V	n.d.	63.50
9	5/f	DBPCFC	CU, RC, IT	III	145.00	0.71
10	14/f	DBPCFC	AE, V, H	IV	n.d.	>100
11	2/f	DBPCFC	CU, C	II	39.00	0.82
12	6/f	DBPCFC	GU, V, S, W	IV	953.00	>100
13	1/m	DBPCFC	GU, AE, R	II	48.00	1.63
14	4/m	DBPCFC	GU, R, W	IV	35.00	0.82
15	4/f	DBPCFC	GU, V	II	542.00	348.00
16	2/f	DBPCFC	GU, W	IV	n.d.	0.85
17	1/f	DBPCFC	V	II	432.00	78.50
18	6/m	OFC	n.d.	unknown	>567	>100
19	5/m	DBPCFC	V, W	IV	231.00	24.50
20	5/f	DBPCFC	GU, RC	III	57.20	3.45
21	3/m	OFC	GU, Cg	III	1985.00	100.00
22	5/m	OFC	GU, AE, RC	III	938.00	84.20
23	3/m	FC	GU, V	II	361.00	70.70
24	10/f	DBPCFC	none	-	n.d.	1.52
25	2/m	DBPCFC	none	-	958.00	3.69
26	2/m	DBPCFC	none	-	n.d.	1.32
27	11/f	DBPCFC	none	-	514.00	2.02
28	6/f	DBPCFC	none	-	n.d.	4.40
29	9/f	OFC	none	-	610.00	2.42
30	6/m	DBPCFC	none	-	n.d.	2.21
31	2/m	DBPCFC	none	-	n.d.	0.51
32	7/f	OFC	none	-	293.00	0.57
33	1/m	OFC	none	-	507.00	1.83
34	2/m	OFC	none	-	1276.00	7.46
35	3/m	DBPCFC	none	-	10.30	1.70
N	n.d.	n.d.	n.d.	-	n.d.	0.02
DLab71S1	n.d.	n.d.	n.d.	-	n.d.	2.75
DLab72S1	n.d.	n.d.	n.d.	-	n.d.	4.33

N, serum from a non-allergic control; *age at time of blood sampling; f, female; m, male; n.d., not determined; DBPCFC, double-blind placebo-controlled food challenge; SBCFC, single-blind controlled food challenge; OFC, open food challenge; FC, food challenge (not further specified); AE, angioedema; C, conjunctivitis; Cg, coughing; CU, contact urticaria; GU, generalized urticaria; H, hoarseness; IT; itching throat; P, pruritus; RA, respiratory arrest; RC, rhinoconjunctivitis; R, rhinitis; S, stridor; V, vomiting; W, wheezing; #severity grading according to the grading system developed by Sampson (Sampson 2003); ‡ determined by ImmunoCAP™.

4.1.2 Generation and physicochemical characterization of recombinant peanut allergens

cDNA sequences (appendix Figure A3-A5) encoding mature Ara h 1, Ara h 2.01 and Ara h 2.02 were successfully cloned into expression vector pPICZαA and subsequently integrated into *Pichia pastoris* X-33 genome as confirmed by DNA-sequencing and PCR analysis, respectively

(data not shown). Both Ara h 2 isoforms could be successfully expressed using *Pichia pastoris*. However, despite using multiple Ara h 1 recombinant clones, it was not possible to express Ara h 1 using *Pichia pastoris*. A potential low expression level could be excluded as verified by the absence after concentrating the cell culture supernatant. In addition, the absence of Ara h 1 in yeast cell culture supernatant was confirmed by immunoblot analysis using anti-His-tag antibody and anti-Ara h 1 (PN-t) antibody (data not shown). As a substitute, rAra h 1 used in this PhD project was kindly provided by Dr. Jonas Lidholm from Thermo Fisher Scientific. It was an *E. coli*-expressed recombinant protein used for commercial ImmunoCAP™ analysis.
For both Ara h 2 isoforms an expression strategy was developed using protease inhibitor solution as supplement during expression to reduce proteolysis. Both full-length proteins were secreted into cell culture supernatant and purified via their His-tag using IMAC and, in the case of rAra h 2.01, via additional SEC. The identity of rAra h 1 and both rAra h 2 isoforms was confirmed by mass spectrometry analysis. Sequence coverages for rAra h 1, rAra h 2.01 and rAra h 2.02 of 46.8%, 41.6% and 39.8% were determined, respectively (Table 15). Furthermore, mass spectrometry analysis identified the sequence of rAra h 1 as the sequence of clone P41B.

Table 15: Identity confirmation of recombinant peanut proteins by mass spectrometry analysis.

Protein	Accession number[#]	PLGS protein score	Number of identified peptides	Sequence coverage	Mass error*
rAra h 1	P43238	3292	30	46.8%	8.1
rAra h 2.01	PEI077	2053	7	41.6%	11.2
rAra h 2.02	PEI129	6452	9	39.8%	6.1

[#]P, UniProt accession number; PEI, internal accession number; *precursor RMS mass error [ppm].

Purity and stability of recombinant proteins was assessed on Coomassie-stained SDS-PAGE. Figure 2A, shows suitable purity of all recombinant peanut protein preparations. Additional bands in the lower molecular weight range in both Ara h 2 isoform preparations were identified by MS as degradation products of the respective isoform. Secondary structure was analyzed using CD spectroscopy (Figure 2B). Far-UV CD-spectrum of rAra h 1 showed a maximum at ~196 nm and a minimum at ~208 nm similar to the published CD-spectrum of Ara h 1 extracted from raw peanuts (Nesbit et al. 2012). However, the calculated hydrodynamic radius (R_H) of 11.2 ± 3 nm indicated a strong aggregation behavior of rAra h 1. The CD spectra of both recombinant Ara h 2 isoforms were comparable to published data and showed characteristics typical for α-helical proteins: two minima at ~208 and ~222 nm and one maximum at ~193 nm (Lehmann et al. 2003). The calculated R_H of rAra h 2.01 and rAra h 2.02

was 2.4 ± 0.2 nm and 2.6 ± 0.5 nm, respectively, suggesting that both proteins appeared as monomers and did not form aggregates in solution. Both isoforms showed comparable physicochemical characteristics and were thus suitable for a direct comparison of their IgE-binding capacity and their diagnostic value that is not impaired by potential differences in their IgE binding as caused by physicochemical differences.

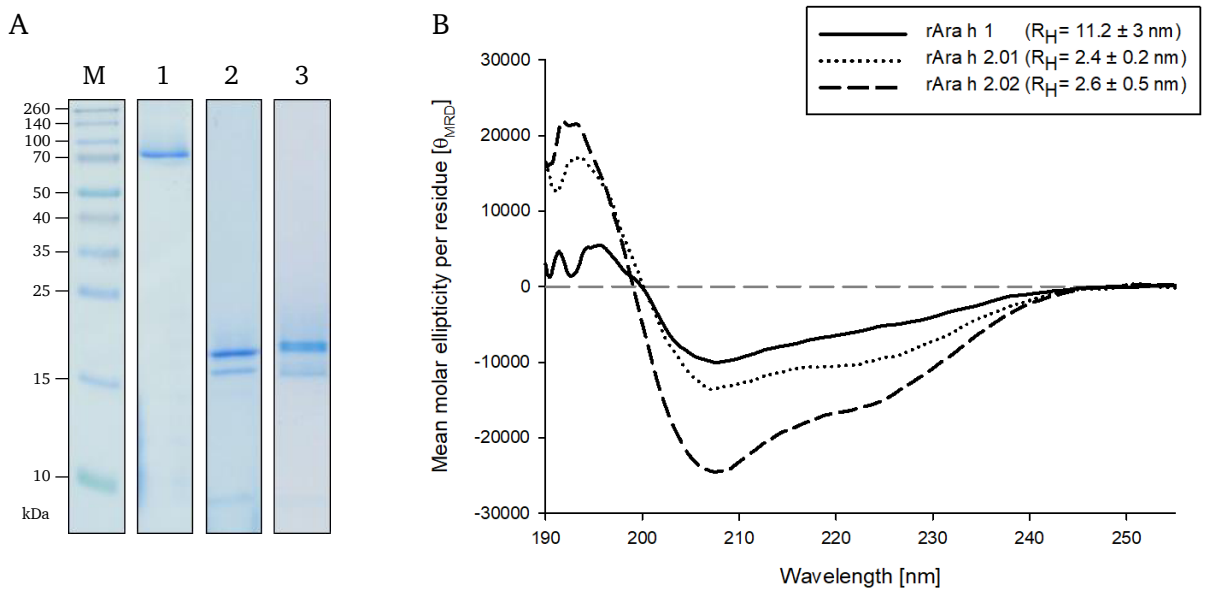


Figure 2: Purity and physicochemical characterization of rAra h 1, rAra h 2.01 and rAra h 2.02.

(A) Coomassie-stained SDS-PAGE of rAra h 1 (lane 1; 3 μg/cm), rAra h 2.01 (lane 2; 2 μg/cm) and rAra h 2.02 (lane 3; 2 μg/cm). Protein samples were analyzed under reducing conditions. M, Spectra™ Multicolor Broad Range Protein Ladder. (B) Far-UV CD-spectra (190-255nm) of rAra h 1 (solid line), rAra h 2.01 (dotted line) and rAra h 2.02 (dashed line). Samples were measured at 2 μM, 3 μM and 3 μM, respectively. The inset depicts the hydrodynamic radius (R_H) ± SD.

4.1.3 Diagnostic value of full-length peanut proteins

In the diagnosis of peanut allergy, the ImmunoCAP™ is a commonly used method. Commercially available ImmunoCAP™ uses rAra h 2.01, which has been reported in several studies to have a high, almost perfect diagnostic accuracy in predicting peanut allergy. Therefore, in a first step the sIgE level to rAra h 2.01 of all sera included in the peanut project was determined by ImmunoCAP™ analysis. Determined sIgE levels to rAra h 2.01 are shown in Table 16. Considering the recommended cut-off value of 0.35 kU_A/L, 22/23 (96%) peanut-allergic children and 1/12 (8%) peanut-tolerant children showed a sensitization to rAra h 2.01. This result shows, in accordance with other published studies, a perfect discriminative ability of sIgE to rAra h 2.01 between peanut-allergic and peanut-sensitized but clinically tolerant children. Therefore, the ImmunoCAP™ result should serve as a reference for subsequent

analysis regarding the assessment of the diagnostic value of the self-generated recombinant peanut allergens.

Due to low serum availability sIgE levels to self-generated recombinant Ara h 1, Ara h 2.01 and Ara h 2.02 were not investigated using ImmunoCAP™ but immunoblot analysis combined with densitometric quantification. Figure 3 shows the serum IgE binding of peanut-allergic (patients 1-23) and sensitized but tolerant (patients 24-35) children to rAra h 1 (Figure 3A), rAra h 2.01 (Figure 3B) and rAra h 2.02 (Figure 3C). For quantification, serial dilutions of two sera having each 103.61 kU_A/L Ara h 1 sIgE and 54.1 kU_A/L Ara h 2 sIgE were used for calibration. The quantified sIgE levels of each patient are listed in Table 16. For identifying positive or negative serum IgE binding to recombinant proteins, the limit of detection (LOD) of each immunoblot was defined as 2 x sIgE level (kU_A/L) of the non-allergic control serum N used on the same membrane. Patients' quantified sIgE levels exceeding the respective LOD were considered positive. LOD's for the specific IgE binding to rAra h 1, rAra h 2.01 and rAra h 2.02 were 1.916/2.058 kU_A/L, 2.404/3.392 kU_A/L and 1.610/2.126 kU_A/L (membrane 1/membrane 2), respectively. A LOD of 1.916 kU_A/L (rAra h 1) was determined for patients 24-34 and of 2.058 kU_A/L for the remaining patients. For rAra h 2.01/rAra h 2.02 immunoblot a LOD of 2.404/1.610 kU_A/L was calculated for patients 1-5 and 24-35, and for the remaining patients a LOD of 3.392/2.126 kU_A/L was determined. Considering the respective LOD, rAra h 2.01 and rAra h 2.02 were recognized by 15/23 (65%) and 18/23 (78%) peanut-allergic children, respectively. In contrast, serum IgE of peanut-tolerant children did not bind to any of both Ara h 2 isoforms in immunoblot analysis. A comparison of ImmunoCAP™ and immunoblot showed that rAra 2.01 was highly specific in immunoblot but less sensitive than in ImmunoCAP™ analysis, which was also reflected by the different cut-off levels for positive signals and the lower amount of serum used for immunoblot analysis. In addition, the immunoblot analysis revealed that rAra h 2.02 had a higher sensitivity compared to rAra h 2.01 (65% vs. 78%) while keeping the specificity (100%). In general it could be shown that in peanut-allergic children sensitized to rAra h 2.01 or rAra h 2.02, the full-length protein (filled triangle Figure 3B and 3C) showed compared to its degradation fragment (open triangle Figure 3B and 3C) a stronger IgE-binding capacity in immunoblot analysis. Moreover, serum IgE of 11/23 (48%) peanut-allergic children and 1/12 (8%) tolerant children bound to rAra h 1 highlighting that rAra h 1 had in this peanut study population the lowest sensitivity (48%) and specificity (92%) of all investigated recombinant allergens in immunoblot analysis.

Generally, 10/23 (43%) peanut-allergic children (patients 1, 6, 10, 12, 15, 17, 18, 21-23) showed a polysensitization to all investigated recombinant proteins. Due to a higher sensitivity of the sIgE detection using ImmunoCAP™ analysis, Ara h 2 sensitizations of patients 2, 4, 13,

14 and 16 could only be detected using ImmunoCAP™ analysis. Moreover, the identified sensitization to rAra h 2.01 in ImmunoCAP™ analysis of patients 5 and 20 could only be reproduced with rAra 2.02 in immunoblot analysis. In contrast, patient 11 showed a sensitization below cut-off (< 0.35 kU_A/L) in ImmunoCAP™ analysis, which was identified as positive, albeit weak, in immunoblot analysis.

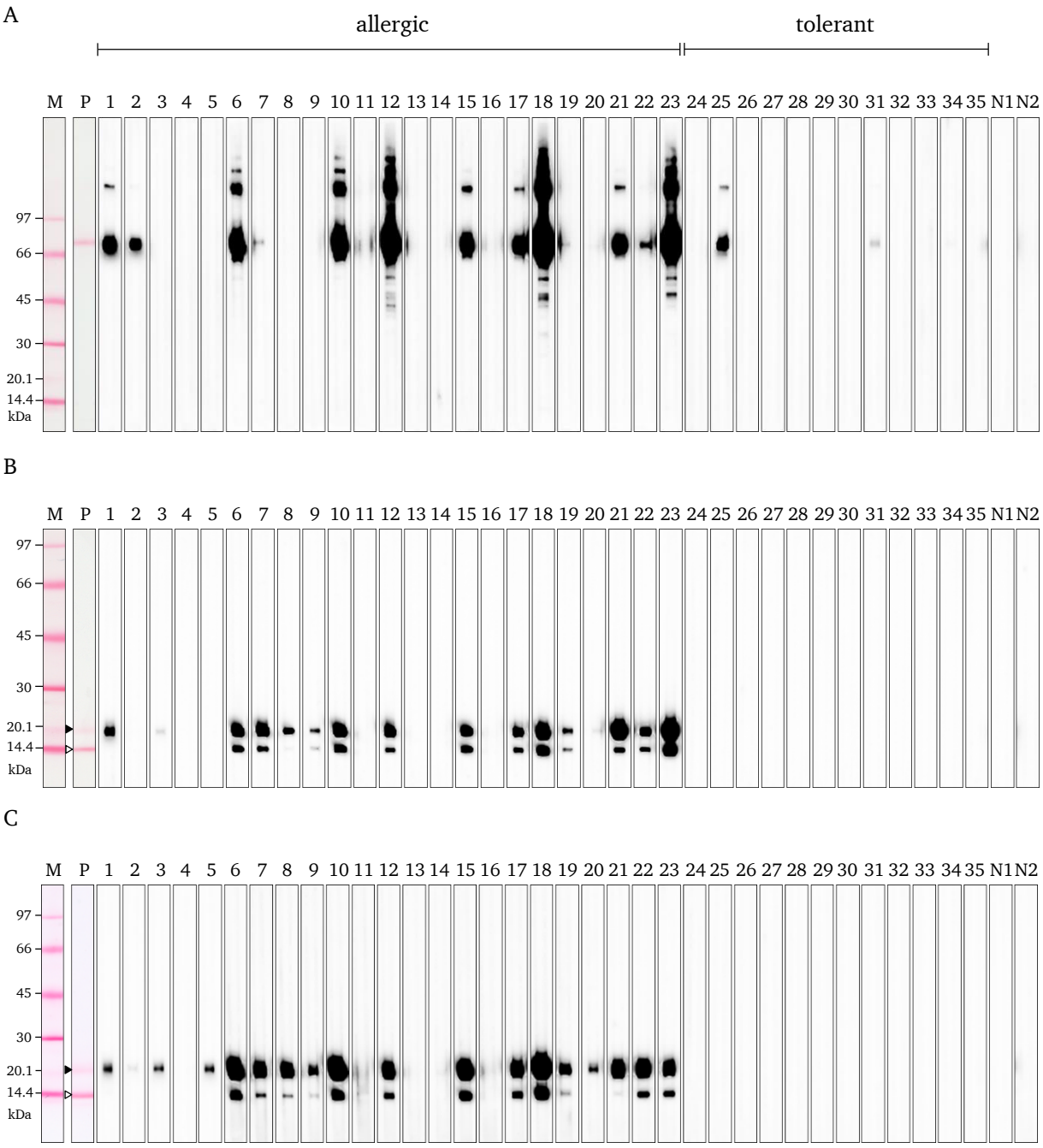


Figure 3: Serum IgE binding of peanut-allergic and sensitized but tolerant children to rAra h 1, rAra h 2.01 and rAra h 2.02.

IgE binding of sera from peanut-allergic children (lane 1-23, patients' sera 1-23 according to Table 14) and tolerant children (lane 24-35, patients' sera 24-35 according to Table 14) to rAra h 1 (A), rAra h 2.01 (B) and rAra h 2.02 (C). Recombinant proteins were analyzed under reducing conditions. IgE binding to each protein after 30 sec exposure is shown. Filled triangles indicate full-length rAra h 2.01 and rAra h 2.02; open triangles indicate degradation products of the respective isoform. M, low-molecular weight marker; P, total protein stain with Ponceau S; N1 and N2, serum from a non-allergic subject on membrane 1 and 2, respectively.

Table 16: Specific IgE levels of peanut-allergic and sensitized but tolerant children.

Peanut-allergic (patients 1-23) and sensitized but tolerant children (patients 24-35). N1 and N2, non-allergic control serum on membrane 1 and 2, respectively; DLab71S1 and DLab72S1, non-allergic controls for microarray analyses.

Patient no.	Peanut sIgE (kU_A/L)‡	rAra h 2.01 sIgE (kU_A/L)‡	rAra h 2.01 sIgE (kU_A/L)†	rAra h 2.02 sIgE (kU_A/L)†	rAra h 1 sIgE (kU_A/L)†
1	28.80	15.00	20.382	7.274	9.520
2	12.80	2.64	(1.633)	(1.452)	5.733
3	23.90	7.99	2.547	5.946	(0.928)
4	4.22	1.02	(1.445)	(0.826)	(0.902)
5	1.98	2.16	(1.351)	6.417	(1.066)
6	>100	>100	29.270	46.879	23.845
7	14.10	31.30	27.196	24.789	(0.943)
8	63.50	71.70	9.674	23.561	(0.746)
9	0.71	2.48	5.216	11.404	(0.879)
10	>100	>100	34.891	54.051	27.755
11	0.82	(0.19)	(1.056)	2.754	(0.962)
12	>100	63.80	23.910	29.540	47.675
13	1.63	1.41	(0.869)	(1.686)	(0.836)
14	0.82	0.46	(0.942)	(1.224)	(0.843)
15	348.00	82.40	28.593	48.594	13.258
16	0.85	0.68	(1.107)	(1.419)	(0.824)
17	78.50	27.20	20.411	32.152	12.074
18	>100	38.80	47.074	62.279	66.386
19	24.50	11.40	8.669	16.209	(0.856)
20	3.45	5.12	(1.287)	6.841	(0.942)
21	100.00	>100	47.182	24.818	12.419
22	84.20	67.90	21.284	33.563	2.476
23	70.70	>100	64.958	26.808	53.195
24	1.52	(0.02)	(1.886)	(1.517)	(1.409)
25	3.69	(0.07)	(1.319)	(1.166)	8.794
26	1.32	(0.01)	(1.362)	(1.112)	(0.985)
27	2.02	(0.02)	(1.224)	(1.079)	(1.018)
28	4.40	(0.02)	(1.195)	(0.996)	(0.962)
29	2.42	(0.00)	(1.370)	(0.946)	(0.973)
30	2.21	(0.02)	(1.161)	(1.025)	(1.043)
31	0.51	(0.00)	(1.259)	(0.909)	(1.414)
32	0.57	(0.03)	(1.178)	(0.915)	(1.025)
33	1.83	(0.02)	(1.377)	(0.981)	(1.358)
34	7.46	(0.05)	(1.361)	(1.014)	(1.098)
35	1.70	0.42	(1.465)	(1.042)	(0.780)
N1	0.02	0.00	1.202	0.805	0.958
N2	0.02	0.00	1.696	1.063	1.029
DLab71S1	2.75	0.01	n.d.	n.d.	n.d.
DLab72S1	4.33	0.02	n.d.	n.d.	n.d.

‡ determined by ImmunoCAP™; † determined by immunoblot analysis; n.d., not determined; () sIgE levels in parenthesis are below method cut-off (LOD).

According to Table 16, the sIgE levels to rAra h 2.01 (> cut-off), determined by ImmunoCAP™ analysis, ranged in peanut-allergic children from 0.46 to > 100 kU_A/L (median 21.1 kU_A/L), whereas only one tolerant child showed sIgE of 0.42 kU_A/L. A similar trend was shown for the sIgE levels (> LOD) to rAra h 2.01 and rAra h 2.02, determined by immunoblot analysis, of peanut-allergic children with median values of 23.91 kU_A/L and 24.80 kU_A/L, respectively, and no tolerant child showing a sensitization to rAra h 2.01 or rAra h 2.02. In contrast, rAra h 1 sIgE levels (> LOD) in peanut-allergic children ranged from 2.48 to 66.39 kU_A/L (median 13.26 kU_A/L) and one tolerant child even showed sIgE binding of 8.79 kU_A/L.

Next, it should be investigated in detail whether the densitometric quantification using immunoblot analysis showed a linear correlation with the conventional ImmunoCAP™ analysis. Therefore, the sIgE levels to rAra h 2.01 determined by ImmunoCAP™ analysis (Table 16) were analyzed for their correlation with sIgE levels to rAra h 2.01 and rAra h 2.02 determined by immunoblot analysis (Figure 4A and 4B, respectively). As shown in Figure 4A, in the range between 0.35 kU_A/L and ~40 kU_A/L sIgE to rAra h 2.01, determined by ImmunoCAP™, a linear correlation (R^2=0.7982) to rAra h 2.01 sIgE levels (> LOQ), determined by immunoblot, was observed. LOQ was defined as 2 x LOD resulting in LOQ1/2 (membrane 1/2) of 4.808/6.784 kU_A/L sIgE to rAra h 2.01. A comparable correlation (R^2=0.7717) was found between sIgE to rAra h 2.01 (ImmunoCAP™) and sIgE to rAra h 2.02 (immunoblot) within analogous limits (Figure 4B). LOQ for sIgE to rAra h 2.02 in immunoblot analysis was 3.22/4.252 kU_A/L for membrane 1/2, respectively. The graphs and the trend lines shown in Figure 4A and 4B demonstrate that the quantification of sIgE levels by means of immunoblot analysis was in the mentioned range comparable to the commonly used ImmunoCAP™ analysis. However, ImmunoCAP™ sIgE levels > 40 kU_A/L could not be exactly reproduced using immunoblot analysis caused by a lower dynamic range of the immunoblot analysis.

Moreover, in Figure 4C rAra h 2.01 and rAra h 2.02 sIgE levels quantified by means of immunoblot analysis were plotted against each other. No linear regression could be calculated but the depicted graph shows that for the great majority of peanut-allergic patients with rAra h 2.01 and rAra h 2.02 sIgE levels > LOQ, rAra h 2.02 was the more sensitive isoform with regard to IgE-binding capacity. 9/13 (69%) peanut-allergic patients with sIgE levels > LOQ showed higher sIgE to rAra h 2.02 and only 4/13 (31%) to rAra h 2.01. This finding suggests that rAra h 2.02 may improve measurement sensitivity when used as diagnostic reagent.

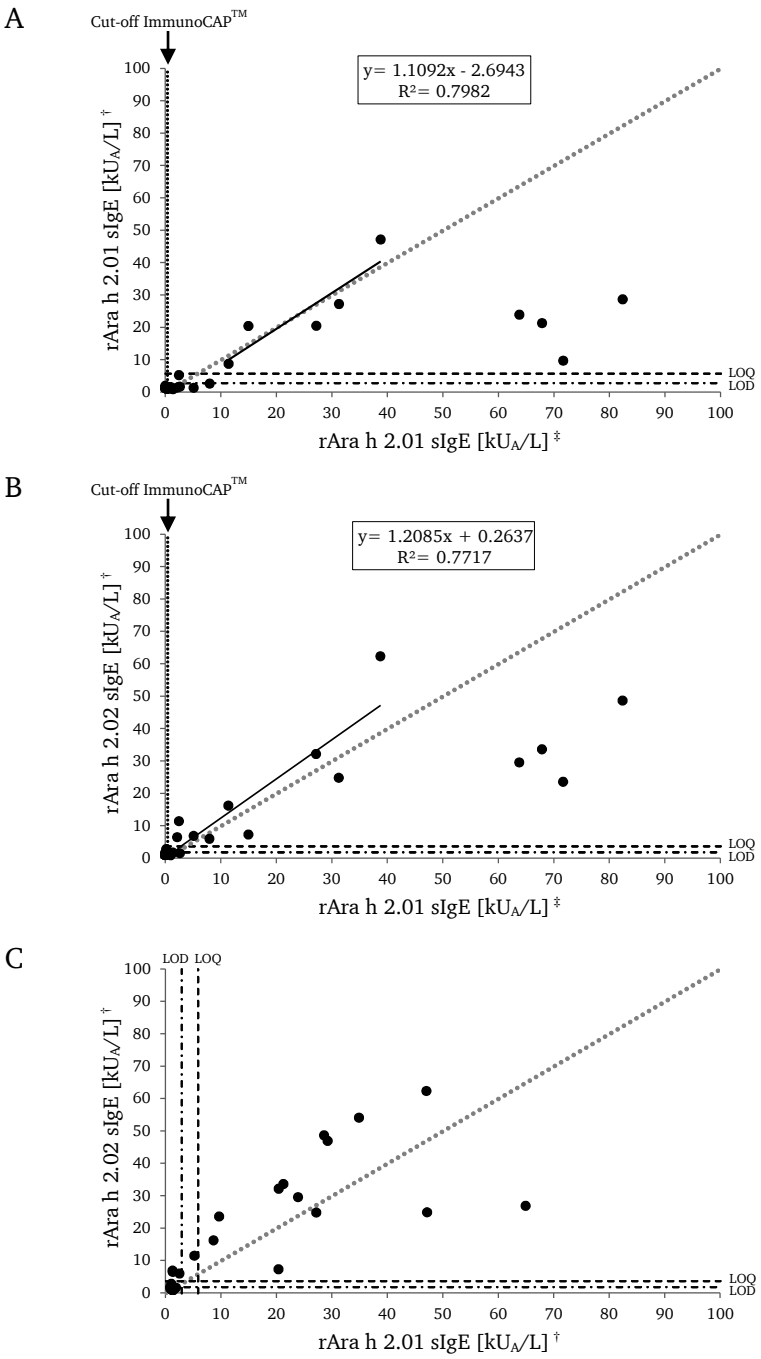


Figure 4: Correlation analysis of sIgE levels quantified by immunoblot analysis and sIgE levels determined by ImmunoCAP™ analysis.

Correlation between rAra h 2.01 sIgE levels determined by ImmunoCAP™ analysis (‡) and sIgE levels to rAra h 2.01 (A) and rAra h 2.02 (B) quantified by immunoblot analysis (†). (C) Correlation between rAra h 2.01 sIgE levels (†) and rAra h 2.02 sIgE levels (†). In (A) and (B) a linear trend line (black line) was drawn through rAra h 2.01 (†) and rAra h 2.02 (†) sIgE levels > LOQ, respectively, and rAra h 2.01 (‡) sIgE levels ≥ 0.35 kU_A/L and ≤ 40 kU_A/L. For simplification in (A)-(C), mean LOD (dashed and single dotted line) and mean LOQ (2 x LOD; dashed line) of LOD1/2 and LOQ1/2 are depicted, respectively. rAra h 2.01 sIgE levels (‡) > 100 kU_A/L were not depicted in (A) and (B). The gray dotted diagonal line represents a perfect correlation (100% correlation with a gradient of 1). R^2, coefficient of determination.

Finally, in order to determine and compare the diagnostic value of the investigated peanut proteins, an ROC curve analysis was performed (Figure 5). To obtain plausible results, the sIgE levels quantified by immunoblot analysis were subtracted by two times the respective sIgE level of the non-allergic control N1 or N2 prior to ROC curve analysis. As otherwise, unexpected high areas under the ROC curve (AUC) were obtained. Furthermore, this step enabled a better adaptation of the ROC curve results with the determined frequency of IgE binding to the respective recombinant proteins. As a reference for ROC curve analysis, the rAra h 2.01 sIgE levels determined by ImmunoCAP™ analysis (Table 16) were used, because most publications use ImmunoCAP™ analysis for determining the sIgE levels, and in this study the ImmunoCAP™ analysis using Ara 2.01 performed almost perfectly.

In general, the higher the AUC, the better the overall diagnostic accuracy of the parameter. sIgE to rAra h 2.01 determined by ImmunoCAP™ analysis showed with an AUC of 1.00 a perfect discrimination between peanut-allergic and peanut-tolerant children within this study population. Using a cut-off of 0.35 kU_A/L a sensitivity of 96% and a specificity of 92% could be observed. For sIgE to peanut extract, also determined by ImmunoCAP™ analysis, an AUC of 0.79 was calculated.

The sIgE to rAra h 2.01 and rAra h 2.02 quantified by means of immunoblot analysis revealed an AUC of 0.75 and 0.86, respectively. In contrast, sIgE to rAra h 1 showed with an AUC of 0.51 the worst performance of all three proteins investigated by immunoblot analysis. Moreover, based on immunoblot analysis, sIgE to rAra h 2.01 reached, at a specificity of 100%, a sensitivity of 65%. Compared to rAra h 2.01, sIgE to rAra h 2.02 showed at a specificity of 100% a higher sensitivity (78%).

In summary, the results presented in this chapter show that rAra h 2.01 in the ImmunoCAP™ analysis enables a perfect discrimination between peanut-allergic and sensitized but tolerant children. In immunoblot analyses, rAra h 1 showed the worst performance compared to rAra h 2.01 and rAra h 2.02, with rAra h 2.02 exceeding rAra h 2.01 with regard to sensitivity. Therefore, it is likely that rAra h 2.02, when used as reagent in ImmunoCAP™ analysis, may additionally improve ImmunoCAP™ performance. At least with regard to measurement sensitivity, as shown for patient 11 who was negative in ImmunoCAP™ analysis but positive (albeit weak) in immunoblot analysis.

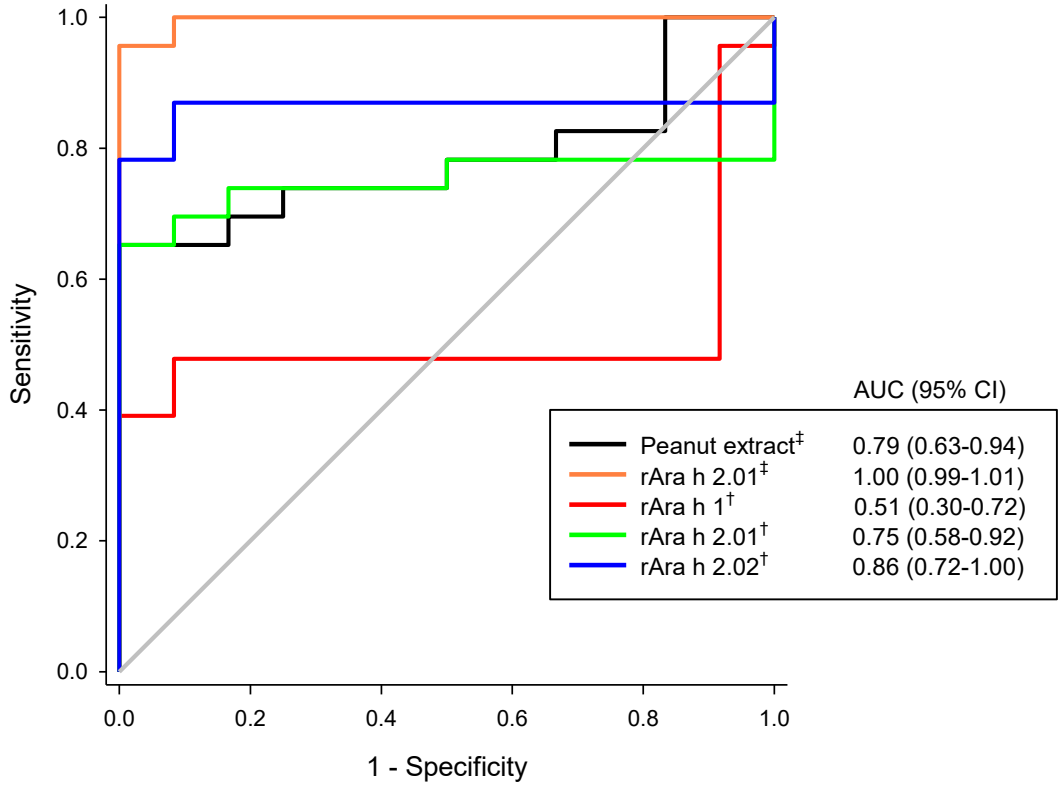


Figure 5: Receiver operating characteristic (ROC) curve analysis of sIgE to peanut extract and full-length recombinant peanut proteins.

ROC curves of sIgE to peanut extract (black), rAra h 2.01 (orange) determined by ImmunoCAP™ analysis (‡) and to rAra h 1 (red), rAra h 2.01 (green) and rAra h 2.02 (blue) quantified by immunoblot analysis (†). For ROC curve analysis, the sIgE levels quantified by means of immunoblot analysis were subtracted by two times the respective sIgE level of the non-allergic control serum. ImmunoCAP™ sIgE values > 100 kU_A/L were set to 100 kU_A/L for ROC curve analysis. The gray diagonal line (AUC 0.5) represents a test without discriminatory ability. AUC, area under the ROC curve; CI, confidence interval.

4.1.4 Differences in IgE binding at the peptide level

In the next analysis step, peanut-allergic and sensitized but tolerant children were analyzed for their differences in IgE binding at the linear peptide level. Therefore, overlapping peptides covering the entire sequences of Ara h 1, Ara h 2.01 and Ara h 2.02 were synthesized. Peptide microarrays were generated and individually probed with patient sera. After immunodetection, peptide signal intensities were recorded and transformed into Z-scores. Proline hydroxylation of Ara h 2 was additionally investigated using peptides containing a hydroxyproline in the DPYSP^{OH}S motif.

Starting with Ara h 1, Figure 6 illustrates the procedure from spotting to IgE immunodetection. In Figure 6A the spotting layout of Ara h 1 peptide microarrays is shown. For simplification just one out of two array segments is shown. In total, 148 peptides comprised the full-length

sequence of Ara h 1. To minimize the influence of outliers, every peptide was spotted in quadruplicate. Controls composed of biotinylated non-allergen related peptides were included for simplifying array analysis. After spotting, Coomassie-staining of randomly chosen array slides verified proper spotting of all peptides (Figure 6B). As an example, the serum IgE binding of a peanut-allergic child (patient 21) is shown in Figure 6C (for more details see Figure A13 in the appendix). The depicted IgE immunodetection showed good accordance between the two array segments regarding the IgE-binding pattern. Moreover, this patient showed a strong IgE binding to the peptides of Ara h 1 with some peptide regions showing maximum IgE binding.

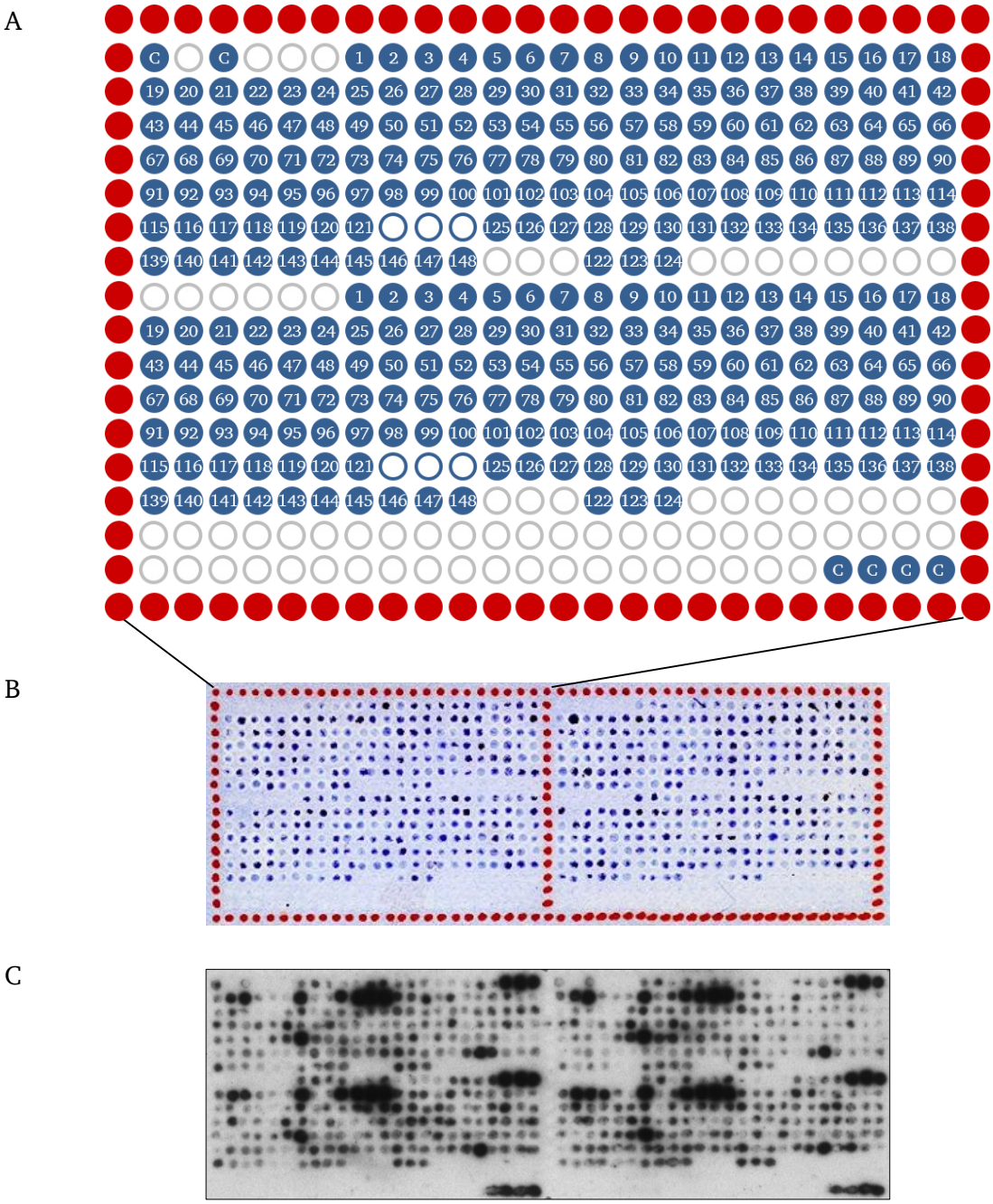


Figure 6: Spotting layout, Coomassie-staining and immunodetection of Celluspot™ multipeptide microarray of Ara h 1.

(A) Spotting layout of Ara h 1 multipeptide microarray. For simplification, only the left segment of the multipeptide microarray is shown. Full-length sequence of Ara h 1 is represented by 148 peptides that were spotted (each 0.04 μl) in duplicate on each segment leading to quadruplicate peptide presentation on the whole array. Biotinylated control peptides, abbreviated by a "C", were spotted in duplicate in different dilutions (top left and bottom right on each array element) and were used as position markers. Gray empty spots represent blank spots composed of peptide printing buffer (DMSO). Blue empty spots represent internal control peptides not relevant for peptide analysis. (B) Successful spotting was verified by staining with Coomassie Brilliant Blue G250. (C) IgE immunodetection using serum of a peanut-allergic child (patient 21) after 30 sec exposure.

In order to compare the signal intensities of different microarrays, the signal intensity of every peptide was transformed into a Z-score. The transformation of signal intensities into Z-scores enables inter-array comparability by reducing the influence of outliers, by correcting the background noise and by considering the variation of the analyzed array. In the next step, non-specific IgE binding to peptides was removed by subtracting from the Z-score of each peptide the maximum Z-score of the same peptide probed with control serum from non-allergic subjects. As control sera, serum N and DLab71S1 were used. IgE binding to a peptide was considered positive, when its Z-score exceeded 2 after control subtraction. Using 2 sigma yielded a probability of more than 95% of positivity for a truly positive signal (Mitchell and Jolley 2013). The calculated Ara h 1 peptide Z-scores of controls (including maximum Z-score) and of allergic and tolerant patients after control subtraction are listed in Table A1-A6 in the appendix.

In order to identify differences in IgE binding between peanut-allergic and tolerant patients, for every peptide of Ara h 1 the frequency of recognition by the peanut-allergic patients was calculated and compared with the frequency of recognition by the tolerant patients (Figure 7).

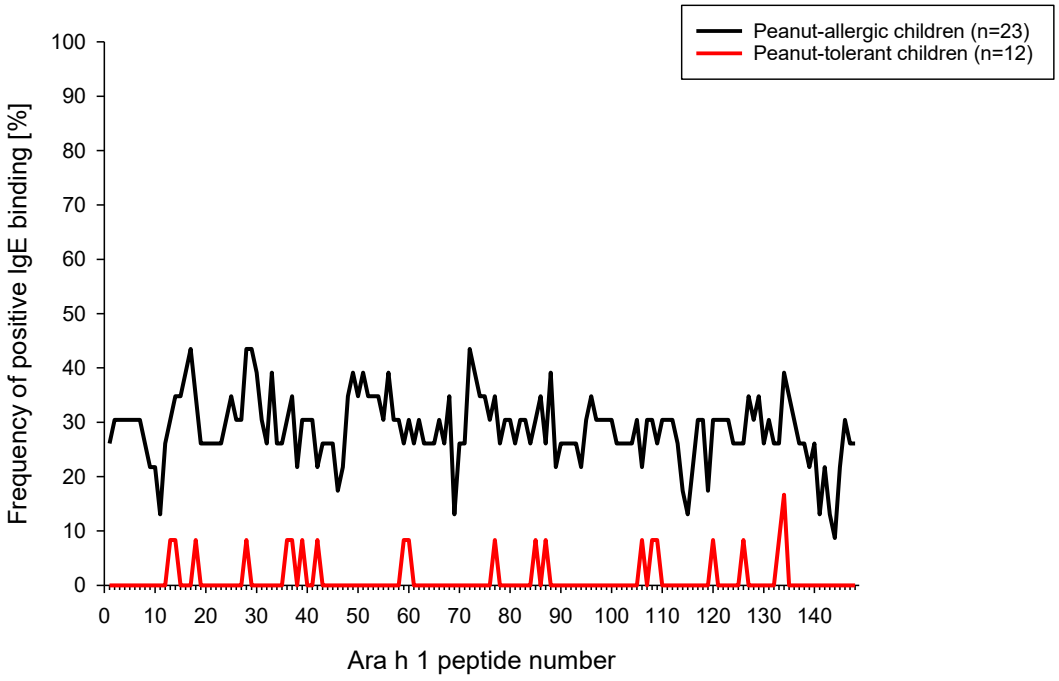


Figure 7: IgE-binding frequencies to peptides of Ara h 1 of peanut-allergic and tolerant children.

Comparison of the IgE-binding frequencies of peanut-allergic (black) and tolerant (red) children to peptides of Ara h 1. Serum IgE binding to a peptide was considered positive if Z-score was > 2.

As shown in Figure 7, peptides of Ara h 1 were recognized by peanut-allergic as well as by tolerant patients. However, peanut-allergic patients showed higher IgE-binding diversity and bound more frequently to peptides of Ara h 1 compared to tolerant patients. The maximum IgE-

binding frequency in peanut-allergic patients was 43% vs. 17% in peanut-tolerant children. Overall, 13/23 (57%) peanut-allergic children and 3/12 (25%) peanut-tolerant children showed an IgE binding to at least one peptide of Ara h 1 (Table 17). Serum IgE from peanut-allergic patients bound between 0 to 147 peptides (median 2), whereas peanut-tolerant patients recognized 0-18 peptides (median 0).

In general, microarray data showed a good correlation with immunoblot data. Except for patients 4, 7, 31 and 34, who only showed an IgE binding in microarray analysis, serum IgE binding to rAra h 1 in immunoblot analysis was in complete accordance with IgE binding to Ara h 1 peptides in microarray analysis. For patients 7 and 31 a very weak IgE binding to rAra h 1 was visible which, however, was below the LOD and was therefore considered negative.

Table 17: Number of positive serum IgE-bound peptides of Ara h 1, Ara h 2.01 and Ara h 2.02.

Positive peptide IgE binding was defined if the Z-score exceeds 2 after control subtraction.

Patient No.	Number of IgE-bound peptides of Ara h 1	Number of IgE-bound peptides of Ara h 2.01_P	Number of IgE-bound peptides of Ara h 2.01_Hyp	Number of IgE-bound peptides of Ara h 2.02_P	Number of IgE-bound peptides of Ara h 2.02_Hyp
1	11	4	6	4	8
2	1	0	0	0	0
3	0	1	6	1	9
4	65	0	0	0	0
5	0	0	0	0	0
6	113	5	6	7	9
7	5	4	7	4	10
8	0	4	6	5	9
9	0	0	1	0	4
10	147	25	25	31	31
11	0	0	0	0	0
12	139	5	6	6	9
13	0	0	0	0	0
14	0	0	0	0	0
15	46	9	9	11	12
16	0	0	5	0	7
17	140	2	7	2	10
18	141	14	14	20	20
19	0	0	0	0	0
20	0	0	4	0	6
21	135	10	10	15	15
22	2	5	5	6	8
23	35	7	7	10	10
24	0	0	0	0	0

25	2	0	0	0	0
26	0	0	0	0	0
27	0	0	0	0	0
28	0	0	0	0	0
29	0	0	0	0	0
30	0	0	0	0	0
31	1	0	0	0	0
32	0	0	0	0	0
33	0	0	0	0	0
34	18	0	0	0	0
35	0	0	0	0	0

Ara h 2.01_P, peptides 9-14 of Ara h 2.01 contain a proline residue in the DPYSPS motif; Ara h 2.01_Hyp, peptides 9-14 of Ara h 2.01 contain a hydroxylated proline residue in the DPYSP^{OH}S motif; Ara h 2.02_P, peptides 9-17 contain a proline residue in the DPYSPS motif; Ara h 2.02_Hyp, peptides 9-17 contain a hydroxylated proline residue in the DPYSP^{OH}S motif.

Next, peanut-allergic and tolerant children were analyzed for their differences in IgE binding to peptides representing full-length mature Ara h 2.01 and Ara h 2.02. Both sequences share high sequence identity (91%) as shown in Figure 8. The differences are shown in red and comprise two amino acid substitutions and an insertion of 12 amino acids in the isoform Ara h 2.02. This insertion contains a further DPYSPS motif leading to the presence of three instead of two DPYSPS motifs in Ara h 2.02. Furthermore, it is reported that natural Ara h 2.01 and Ara h 2.02 undergo site-specific post-translational hydroxylation leading to DPYSPS motifs with the second proline being hydroxylated (Figure 8, blue).

```
Ara h 2.01   1    RQQWELQGDRRCQSQLERANLRPCEQHLMQKIQRDEDSYERDPYSPSQDPYSPS------   54
Ara h 2.02   1    RQQWELQGDRRCQSQLERANLRPCEQHLMQKIQRDEDSYGRDPYSPSQDPYSPSQDPDRR   60

Ara h 2.01   55   ------PYDRRGAGSSQHQERCCNELNEFENNQRCMCEALQQIMENQSDRLQGRQQEQQF   108
Ara h 2.02   61   DPYSPSPYDRRGAGSSQHQERCCNELNEFENNQRCMCEALQQIMENQSDRLQGRQQEQQF   120

Ara h 2.01   109  KRELRNLPQQCGLRAPQRCDLDVESGGRDRY  139
Ara h 2.02   121  KRELRNLPQQCGLRAPQRCDLEVESGGRDRY  151
```

Figure 8: Sequence alignment of Ara h 2.01 and Ara h 2.02.

Mature full-length sequences of Ara h 2.01 and Ara h 2.02 were aligned using BLAST® (NCBI, Bethesda MD, USA). Differences in amino acid sequence are shown in red, DPYSPS motifs are underlined and proline residues undergoing post-translational hydroxylation in natural Ara h 2 are shown in blue.

Figure 9A shows the spotting layout of one segment of the Ara h 2 multipeptide microarray. Ara h 2.01 and Ara h 2.02 were composed of 32 and 35 peptides, respectively. The individual

peptide sequences are listed in the appendix (e.g. Table A8 and A25). Peptides shared by both isoforms were just spotted once for both isoforms (half dark blue, half light blue spots Figure 9A). In order to analyze the influence of proline hydroxylation on IgE binding, peptides containing hydroxyproline residues were additionally investigated on each array. Peptides containing hydroxylated proline residues are marked with an asterisk (Ara h 2.01: peptides 9-4; Ara h 2.02: peptides 9-17). Again, Coomassie-staining confirmed the presence of all peptide spots on the array slide (Figure 9B).

As an example, the immunodetection using serum IgE from allergic patient no. 18 is shown in Figure 9C. This patient showed a strong IgE binding to peptides that contain the DPYSPS motif (Ara h 2.01: peptides 9-13; Ara h 2.02: peptides 9-16) or at least a partial amino acid sequence of it (Ara h 2.01: peptide 14; Ara h 2.02: peptide 17). The proline hydroxylation in the mentioned peptides led to a further increase in IgE binding in this patient. The increase in the IgE-binding capacity due to the hydroxylation could be observed for the great majority of the allergic children (see appendix Figure A15).

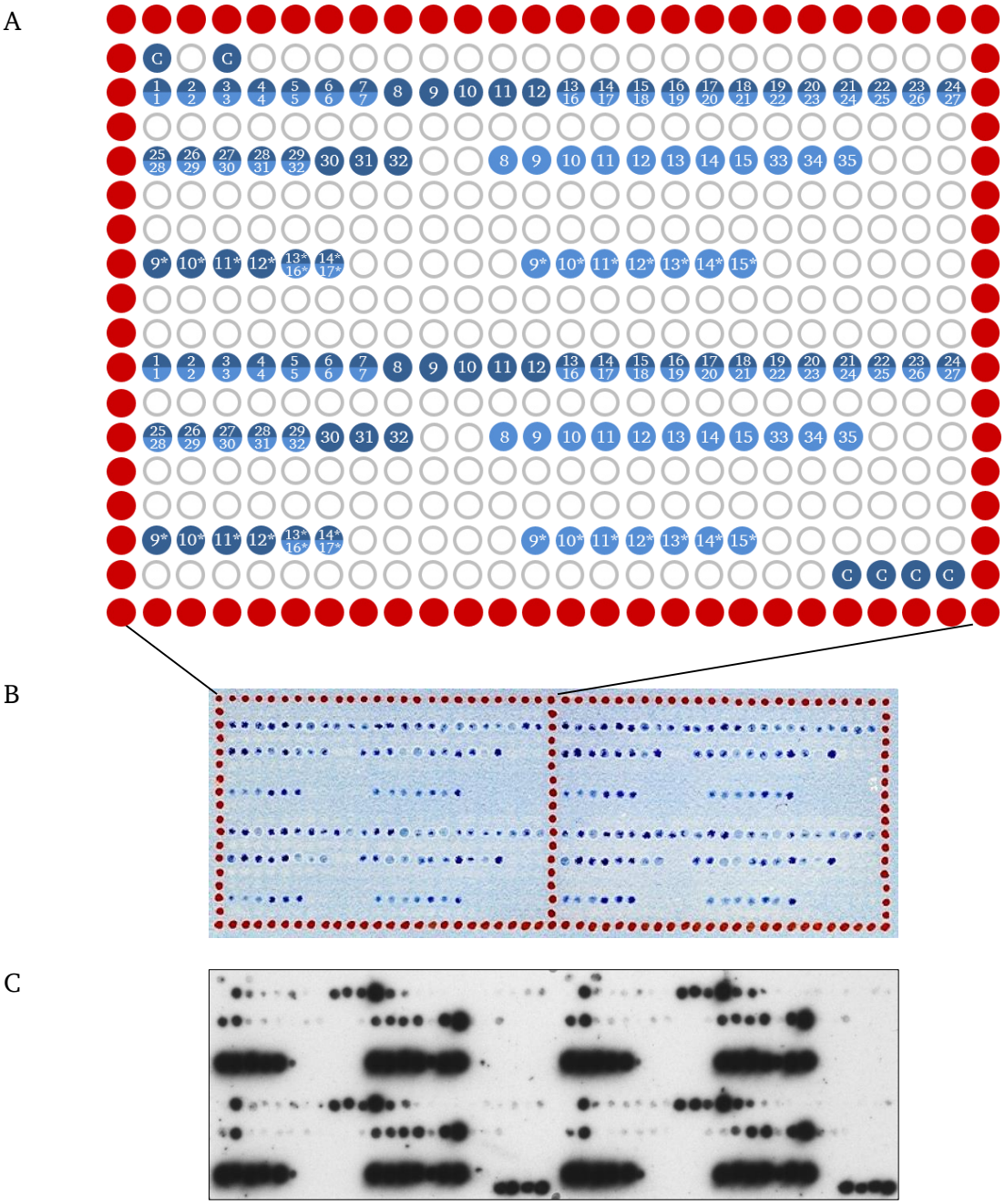


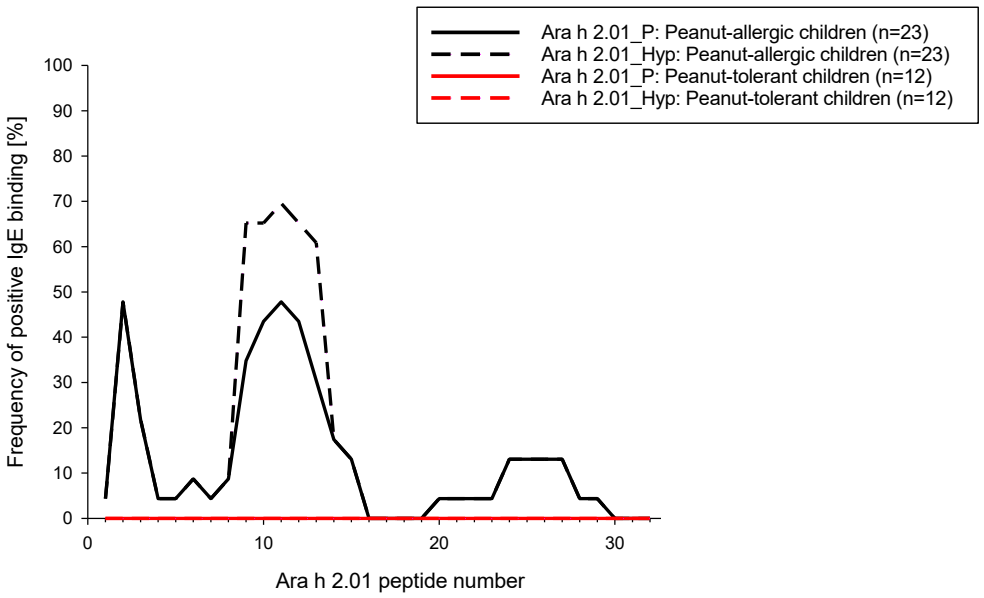
Figure 9: Spotting layout, Coomassie-staining and IgE immunodetection of Celluspot™ multipeptide microarray displaying Ara h 2.

(A) Spotting layout of Ara h 2 multipeptide microarray. For simplification, only the left segment of the multipeptide microarray is shown. Dark and light blue spots represent unique peptides either found in Ara h 2.01 or Ara h 2.02, respectively. Half dark blue, half light blue spots represent peptides shared by both isoforms. Peptide numbers written in dark blue and light blue areas belong to Ara h 2.01 and Ara h 2.02, respectively. Peptides depicted with asterisks contain hydroxyproline residues in comparison to their peptide counterparts depicted without asterisks. In general, Ara h 2.01 and Ara h 2.02 are covered by 32 and 35 peptides, respectively. Biotinylated control peptides, abbreviated by a "C", were spotted in different dilutions (top left and bottom right on each array element) and were used as position markers. Gray empty spots represent blank spots composed of peptide printing buffer (DMSO). (B) Successful spotting was verified by staining with Coomassie Brilliant Blue G250. (C) IgE immunodetection using serum of peanut patient 18 after 30 sec exposure.

For simplification, the full-length sequences of Ara h 2.01 and Ara h 2.02 without site-specific proline hydroxylation will be named as Ara h 2.01_P and Ara h 2.02_P, respectively. Here, peptides 9-14 of Ara h 2.01 and peptides 9-17 of Ara h 2.02 contain a proline residue in the DPYSPS motif. If the mentioned peptides contain a hydroxylated proline residue in the DPYSP^{OH}S motif, both full-length sequences will be named as Ara h 2.01_Hyp and Ara h 2.02_Hyp. The calculated Z-scores (after control subtraction) of every peptide of Ara h 2.01_P, Ara h 2.01_Hyp, Ara h 2.02_P and Ara h 2.02_Hyp are listed in Table A8-A23 and A25-A40 in the appendix. Mentioned tables in the appendix show the peptide Z-scores of all peanut-allergic and tolerant children as well as of controls and their derived maximum peptide Z-scores.

For every peptide of the four investigated Ara h 2 sequences the frequency of recognition by the allergic children and by the tolerant children was calculated. Again, a Z-score > 2 was used for defining positive peptide IgE binding. Figure 10 shows the calculated frequency of positive IgE binding of allergic and tolerant children to peptides of Ara h 2.01_P and Ara h 2.01_Hyp (Figure 10A) and to peptides of Ara h 2.02_P and Ara h 2.02_Hyp (Figure 10B).

A



B

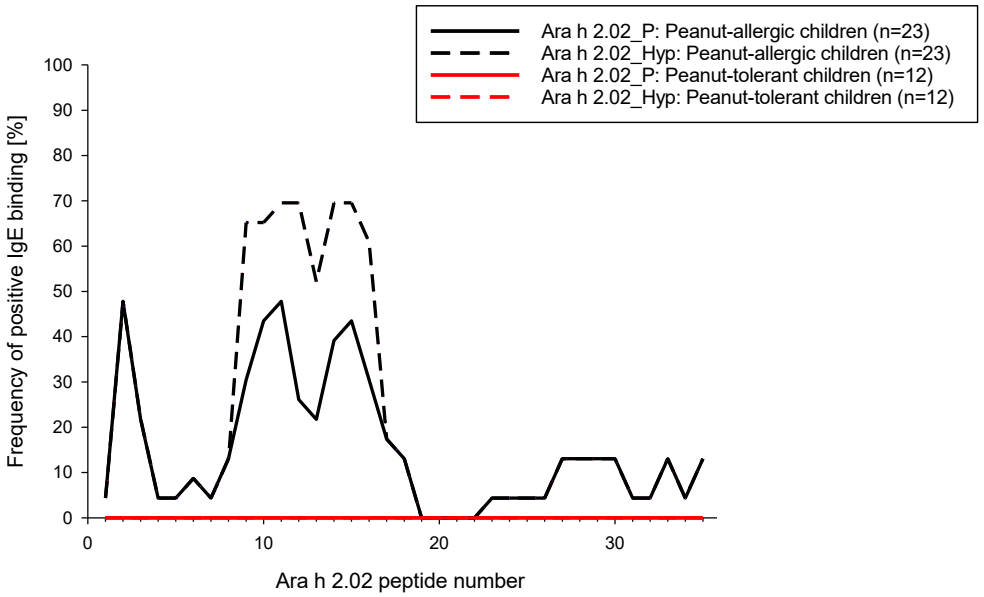


Figure 10: IgE-binding frequencies to peptides of Ara h 2.

Peanut-allergic (black) and tolerant patients (red) were analyzed for their IgE binding frequencies to peptides of Ara h 2.01_P and Ara h 2.01_Hyp (A) and to peptides of Ara h 2.02_P and Ara h 2.02_Hyp (B). IgE-binding frequencies to peptides containing a hydroxyproline are depicted in (A) and (B) as dashed line. Criterion for positive IgE binding was a Z-score > 2.

Comparable to the IgE binding to full-length allergens (Figure 3), only peanut-allergic children showed IgE binding to the peptides of both isoforms (Figure 10). Serum IgE of peanut-tolerant children did not bind to any peptide, even peptides 9-14 of Ara h 2.01_Hyp and peptides 9-17 of Ara h 2.02_Hyp containing hydroxylated proline residues did not bind to serum IgE of tolerant children. In peanut-allergic children, peptides with maximum IgE-binding frequencies can be identified. Peptide 2 located at the amino-terminal part of the protein sequences was

recognized by 11/23 (48%) allergic children. In addition, serum IgE of 11 (48%) peanut-allergic children, with 9 patients also recognizing peptide 2, bound to peptide 11 of Ara h 2.01_P and peptide 11 of Ara h 2.02_P. Peptide 11 of Ara h 2.01_P and of Ara h 2.02_P contained two DPYSPS motifs, respectively (for peptide sequence information see appendix Table A8 and A25). Hydroxylation of proline residues in Ara h 2.01_Hyp and Ara h 2.02_Hyp led to an increase in the maximum IgE-binding frequency. Due to hydroxyproline, 16/23 (70%) peanut-allergic children recognized peptide 11 of Ara h 2.01_Hyp and the same 16 children recognized peptides 11, 12, 14 and 15 of Ara h 2.02_Hyp. Peptides representing the carboxy-terminal part of the protein sequences seemed to be of lower relevance for linear IgE binding.

According to Table 17, serum IgE of 13/23 (57%) peanut-allergic children bound to any peptide of Ara h 2.01_P and Ara h 2.02_P, respectively. The median of IgE-bound peptides in the allergic children was in both sequences 2 peptides. Proline hydroxylation led to an increase in the median number of IgE-bound peptides in Ara h 2.01_Hyp and Ara h 2.02_Hyp with a median number of 6 and 8 peptides, respectively. Overall, 16/23 (70%) of peanut-allergic children showed an IgE binding to at least one peptide of Ara h 2.01_Hyp and Ara h 2.02_Hyp. Serum IgE of patients 9, 16 and 20 bound only to peptides containing a hydroxylated proline residue and not to peptides of Ara h 2.01_P and Ara h 2.02_P. Due to hydroxylation in Ara h 2.01_Hyp and Ara h 2.02_Hyp, 10/23 and 12/23 peanut-allergic children recognized more peptides as in Ara h 2.01_P and Ara h 2.02_P, respectively. Furthermore, hydroxylation was also relevant for IgE binding of patients 3 and 17. Both patients recognized few peptides in Ara h 2.01_P and Ara h 2.02_P, however peptides 9-14 of Ara h 2.01 and peptides 9-17 of Ara h 2.02 were only recognized if they contained hydroxylated proline residues. Moreover, as shown in Figure 9C, hydroxylation led to higher signal intensities of IgE-bound peptides. 11/23 allergic patients showed an IgE binding to the sum of peptides 9-14 of Ara h 2.01_P. Hydroxylation led to an increase in serum IgE binding with 16 patients showing an IgE binding to the sum of peptides 9-14 of Ara h 2.01_Hyp. In 14 of these 16 patients hydroxylation led to higher signal intensities of IgE-bound peptides. The same tendency could be detected for peptides 9-17 of Ara h 2.02_P versus Ara h 2.02_Hyp. Here, the IgE binding to the sum of these peptides increased from 11 to 16 allergic patients and in all (16/16) allergic patients showing a positive IgE binding the hydroxylation led to higher signal intensities of IgE-bound peptides (for more details see appendix Table A8-A23 and A25-A40).

Subsequently, microarray and immunoblot data were compared with regard to their serum IgE binding. As recombinant Ara h 2.01 and Ara h 2.02 cannot undergo proline hydroxylation (see Figure 13), the microarray data of Ara h 2.01_P and Ara h 2.02_P were used for the comparison. It turned out, that serum IgE binding of tolerant children was in both methods in complete

agreement with no detectable IgE binding to either the recombinant isoforms Ara h 2.01/Ara h 2.02 or the derived peptides. Furthermore, except for allergic patients 9 and 19, who only showed IgE binding to Ara h 2.01 in the immunoblot analysis, microarray analysis of Ara h 2.01_P of peanut-allergic patients was in complete accordance with the immunoblot analysis. Moreover, the comparison of serum IgE binding to Ara h 2.02 in immunoblot and microarray analysis revealed that five patients (patients 5, 9, 11, 19 and 20) showed an IgE binding to full-length rAra h 2.02 but not to peptides of Ara h 2.02_P.

4.1.5 Identification of candidate diagnostic peptides

In order to identify peptides with diagnostic specificity for peanut allergy, selection criteria for candidate diagnostic peptides were established. Candidate diagnostic peptides should be exclusively recognized by peanut-allergic children. Furthermore, only positive IgE-bound peptides with high signal intensities in IgE binding were selected. A median Z-score of > 5 should sufficiently ensure to eliminate weak positive IgE-binding peptides. Further, only peptides showing an IgE binding in relation to the full-length allergen were selected. Therefore, inhibition experiments using serum pools were performed and only peptides were further selected which showed an inhibition of their IgE binding by the corresponding full-length protein. In general, sera were pooled based on comparable IgE-binding pattern to the investigated peptides.

To verify the specificity of the IgE binding to Ara h 1-derived peptides, two serum pools were generated and IgE binding was inhibited by the addition of 20.5 μg rAra h 1. Pool 1 was composed of sera from patients 10, 12, 18 and 21, and pool 2 of sera from patients 6, 15, 17, 23. For the verification of the specificity of IgE binding to Ara h 2 peptides, sera from patients 6-8, 10, 12, 15, 18, 21-23 were pooled and preincubated with either 13.5 μg rAra h 2.02, 9.5 μg native or r/a peanut extract.

Candidate peptides of Ara h 1 and Ara h 2 (Ara h 2.01_P/Hyp and Ara h 2.02_P/Hyp) were selected if they showed an inhibition of ≥ 30% of their IgE binding in at least one inhibition experiment. With Ara h 1 as an example, an at least 30% inhibition of peptide IgE binding by rAra h 1 in at least one of the two used serum pools was required to address Ara h 1 specificity. In the case of Ara h 2, peptides were selected that could be inhibited in their IgE binding by either rAra h 2.02, native or r/a peanut extract by at least 30%. Images of the Ara h 1 and Ara h 2 IgE inhibition experiments and calculated Z-scores can be found in the appendix, in Figure A14/Table A7 and Figure A16/Table A24 & Table A41, respectively.

Furthermore, candidate peptides should contain distinct amino acid sequences and represent distinct protein areas and therefore an offset of ≥ 4 peptides was applied between two peptides.

If there were several options, peptides with the highest IgE-binding frequency were selected. Among peptides that showed an identical IgE-binding frequency, those with the highest median Z-score were selected.

Finally, from all preselected peptides fulfilling the above-mentioned criteria, the most frequently recognized peptides (≥ median frequency of preselected peptides) were selected as candidate diagnostic peptides.

By applying these criteria, three peptides of Ara h 1, peptide 17, 75 and 86, could be identified (Figure 11). These three peptides were individually bound by serum IgE from 10 (43%), 8 (35%) and 8 (35%) peanut-allergic children, respectively. In addition, 11/23 (48%) peanut-allergic children recognized at least one of these three peptides in contrast to no tolerant child (Table 18).

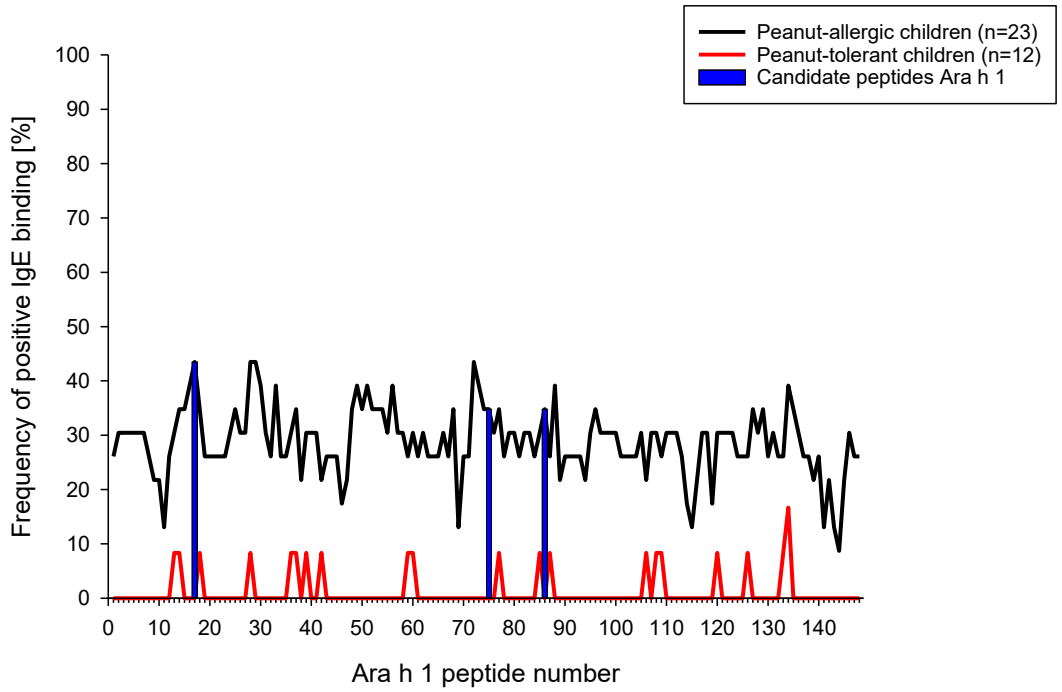


Figure 11: Identified peptides of Ara h 1 fulfilling the selection criteria for candidate diagnostic peptides.

Comparison of the IgE-binding frequencies of peanut-allergic (black) and tolerant (red) children to peptides of Ara h 1. Serum IgE binding to a peptide was considered positive if Z-score was > 2. Identified candidate diagnostic peptides within this study population specific for peanut allergy are shown in blue bars.

Table 18: IgE binding of peanut-allergic and tolerant children to identified candidate diagnostic peptides of Ara h 1.

Three peptides were selected as candidate diagnostic peptides of Ara h 1. X depicts positive serum IgE binding to candidate peptide; - depicts negative serum IgE binding.

Patient No.	Ara h 1 P17	Ara h 1 P75	Ara h 1 P86
1	X	-	-
2	-	-	-
3	-	-	-
4	-	X	X
5	-	-	-
6	X	X	X
7	-	-	-
8	-	-	-
9	-	-	-
10	X	X	X
11	-	-	-
12	X	X	X
13	-	-	-
14	-	-	-
15	X	-	X
16	-	-	-
17	X	X	X
18	X	X	X
19	-	-	-
20	-	-	-
21	X	X	X
22	X	-	-
23	X	X	-
24	-	-	-
25	-	-	-
26	-	-	-
27	-	-	-
28	-	-	-
29	-	-	-
30	-	-	-
31	-	-	-
32	-	-	-
33	-	-	-
34	-	-	-
35	-	-	-

The respective amino acid sequence and the molecular surface presentation of the three selected peptides is shown in Table 19 and Figure 12, respectively. Interestingly, a relatively high content

of charged amino acid residues could be observed in peptide 17 and 86. As peptide 17 is located in Ara h 1 where the structure is not resolved, it could not be depicted in Figure 12. Peptide 75 and peptide 86, as shown in Figure 12, represented two adjacent protein areas.

Table 19: Identified candidate diagnostic peptides of Ara h 1.

Amino acid sequences of identified candidate diagnostic peptides of Ara h 1.

Candidate peptides of Ara h 1	Amino acid sequence
Peptide 17	E-R-T-R-G-R-Q-P-G-D-Y-D-D-D-R
Peptide 75	E-A-A-F-N-A-E-F-N-E-I-R-R-V-L
Peptide 86	V-K-V-S-K-E-H-V-E-E-L-T-K-H-A

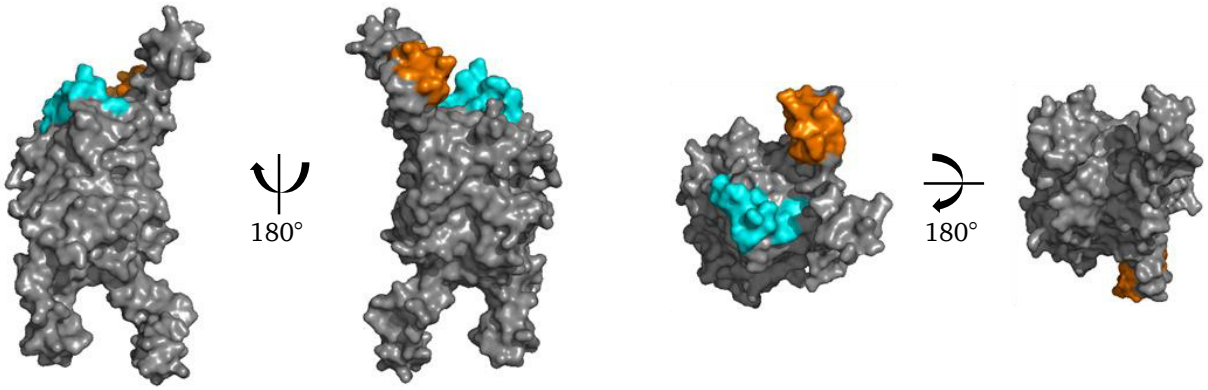


Figure 12: Surface presentation of identified candidate diagnostic peptides of Ara h 1.

Cyan and orange colored amino acids represent identified candidate diagnostic peptide 75 and peptide 86, respectively. Peptide 17, located where the structure of Ara h 1 is not resolved, could not be depicted. Using Ara h 1 pdb 3SMH and PyMOL, 3-D images were generated.

Candidate diagnostic peptides could also be identified of Ara h 2, but at first, the result of the microarray IgE inhibition should be described in more detail.

On the one hand, the inhibition experiment verified the specificity of the peptide IgE binding, since full-length Ara h 2 was able to inhibit IgE binding to peptides. On the other hand, the experiment showed that the recombinant Ara h 2, compared to its natural counterpart, did not undergo proline hydroxylation, which led to the absence of hydroxyproline in the immunodominant DPYSPS motif.

As shown in Figure 13, recombinant Ara h 2.02 (13.5 μg) could inhibit IgE binding to Ara h 2.01_P and Ara h 2.02_P completely. However, IgE binding to peptides containing hydroxylated proline residues (Figure 13, highlighted in red) could be only partially inhibited by rAra h 2.02. In contrast, only natural Ara h 2 in native and reduced/alkylated peanut extract

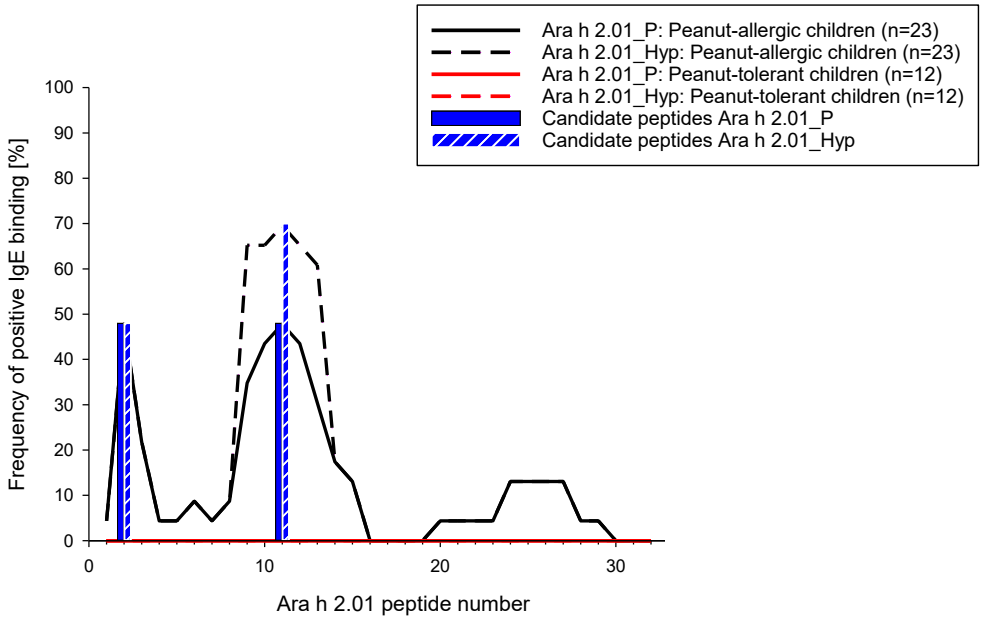
could inhibit IgE binding to these peptides. Taking into account that Ara h 2 accounts for 6-9% of the total peanut protein content, it makes up ~0.6-0.9 μg in each peanut extract. However, this amount was approximately 10-fold lower than the amount of recombinant Ara h 2.02 used in the inhibition experiment, which further supports that recombinant Ara h 2 did not undergo proline hydroxylation and thus cannot completely inhibit IgE binding to Hyp-containing peptides.

Figure 13: Specificity of IgE binding to Ara h 2-derived peptides verified by IgE inhibition experiments. Using rAra h 2.02 (13.5 μg), native peanut extract (9.5 μg) and reduced/alkylated peanut extract (9.5 μg) the specificity of the peptide IgE binding was verified. As reference, serum pool plus protein buffer was used. Peptides containing hydroxylated proline residues are highlighted in red. IgE immunodetections after 30 sec exposure are shown.

Taking into account the mentioned selection criteria for candidate diagnostic peptides, in total four peptide pairs could be identified of the analyzed Ara h 2.01 and Ara h 2.02 sequences (Figure 14A and 14B; blue full bars and blue dashed bars). Blue full bars represent candidate

peptides of Ara h 2.01_P and Ara h 2.02_P. Blue dashed bars depict candidate peptides of Ara h 2.01_Hyp and Ara h 2.02_Hyp. In each Ara h 2 sequence one peptide pair could be identified.

A



B

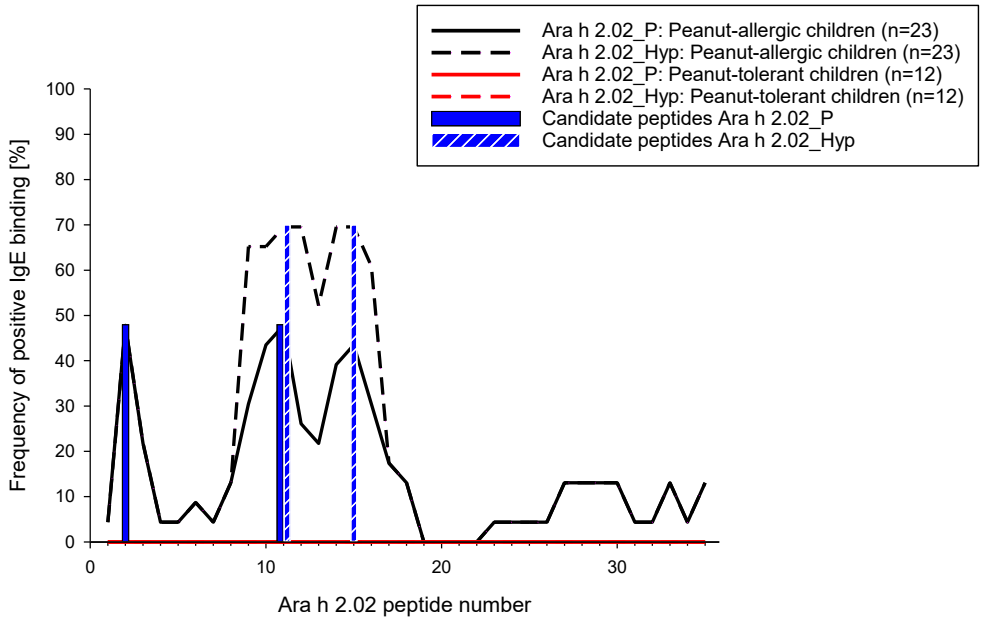


Figure 14: Identified peptides of Ara h 2.01 and Ara h 2.02 fulfilling the selection criteria for candidate diagnostic peptides.

Comparison of the IgE-binding frequencies of peanut-allergic (black) and tolerant (red) children to peptides of Ara h 2.01_P and Ara h 2.01_Hyp (A) and to peptides of Ara h 2.02_P and Ara h 2.02_Hyp (B). IgE-binding frequencies to peptides containing a hydroxyproline are depicted in (A) and (B) as black dashed line. Serum IgE binding to a peptide was considered positive if Z-score was > 2. Identified candidate diagnostic peptides of Ara h 2.01_P (A) and Ara h 2.02_P (B) are shown as blue full bars. Candidate peptides of Ara h 2.01_Hyp (A) and Ara h 2.02_Hyp (B) are shown as blue dashed bars.

In both Ara h 2.01 sequences, Ara h 2.01_P and Ara h 2.01_Hyp, peptide 2 and peptide 11 could be identified as candidate diagnostic peptides. Peptide 2 was in both cases the same peptide being composed of the same amino acids as it was shared by both investigated Ara h 2.01 sequences. Peptide 11 of Ara h 2.01_Hyp contained, compared to peptide 11 of Ara h 2.01_P, hydroxyproline residues instead of proline residues (Table 20). In addition, two peptide pairs could be identified of Ara h 2.02_P and Ara h 2.02_Hyp (Figure 14B). Of Ara h 2.02_P, peptide 2 and 11 could be identified, and of Ara h 2.02_Hyp, peptide 11 and 15. Peptide 2 of Ara h 2.02_P was the same peptide as of Ara h 2.01_P and Ara h 2.01_Hyp (Table 20). Compared to peptide 11 of Ara h 2.02_P, peptide 11 of Ara h 2.02_Hyp contained hydroxylated proline residues. Interestingly, peptide 11 was in all analyzed Ara h 2 sequences the only peptide containing two times the entire DPYSPS motif. The only difference between peptide 11 of Ara h 2.01_P/Hyp and Ara h 2.02_P/Hyp is a glutamine (Q) instead of a proline (P) in Ara h 2.02.

Table 20: Identified candidate diagnostic peptides of Ara h 2.

Amino acid sequences of identified candidate diagnostic peptides of Ara h 2.01_P, Ara h 2.01_Hyp, Ara h 2.02_P and Ara h 2.02_Hyp.

Candidate peptides	Analyzed protein	Amino acid sequence
Peptide 2	Ara h 2.01_P; Ara h 2.01_Hyp; Ara h 2.02_P	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A
Peptide 11	Ara h 2.01_P	R-D-P-Y-S-P-S-Q-D-P-Y-S-P-S-P
Peptide 11	Ara h 2.01_Hyp	R-D-P-Y-S-Hyp-S-Q-D-P-Y-S-Hyp-S-P
Peptide 11	Ara h 2.02_P	R-D-P-Y-S-P-S-Q-D-P-Y-S-P-S-Q
Peptide 11	Ara h 2.02_Hyp	R-D-P-Y-S-Hyp-S-Q-D-P-Y-S-Hyp-S-Q
Peptide 15	Ara h 2.02_Hyp	P-D-R-R-D-P-Y-S-Hyp-S-P-Y-D-R-R

Patients' IgE binding to identified candidate diagnostic peptides is shown in Table 21. According to the selection criteria of candidate diagnostic peptides, serum of no tolerant child showed an IgE binding to any of the four selected peptide pairs. In contrast, serum IgE of 13/23 (57%) peanut-allergic children showed an IgE binding to the peptide pair (peptide 2 plus peptide 11) of Ara h 2.01_P. The same 13 peanut-allergic children also recognized the peptide pair of Ara h 2.02_P (peptide 2 plus peptide 11). Nine of these 13 children recognized both peptides of the selected peptide pair in both analyzed Ara h 2.01_P and Ara h 2.02_P sequences. Individually, peptide 2 and 11 were recognized by 48% (11/23) of peanut-allergic children in both Ara h 2.01_P and Ara h 2.02_P. In Ara h 2.01_Hyp, serum IgE from a total of 16/23 (70%) peanut-allergic children bound to the peptide pair (peptide 2 plus peptide 11). 11 of these 16

patients recognized both peptides and additional five patients recognized solely peptide 11 leading to an increased IgE-binding frequency of 70% (16/23) to peptide 11.

Moreover, both selected peptides of Ara h 2.02_Hyp (peptide 11 plus peptide 15) were bound by serum IgE of 16/23 peanut-allergic patients, in total. Individually, peptide 11 and peptide 15 of Ara h 2.02_Hyp were recognized by 16/23 patients, respectively. These 16 patients were the same patients who also showed an IgE binding to the selected peptide pair of Ara h 2.01_Hyp.

Table 21: IgE binding of peanut-allergic and tolerant children to identified candidate diagnostic peptides of Ara h 2.

Four peptide pairs were selected as candidate diagnostic peptides of Ara h 2.01_P, Ara h 2.01_Hyp, Ara h 2.02_P and Ara h 2.02_Hyp, in total. X depicts positive serum IgE binding to candidate peptide; - depicts negative serum IgE binding.

Patient No.	Ara h 2.01_P P2	Ara h 2.01_P P11	Ara h 2.01_Hyp P2	Ara h 2.01_Hyp P11	Ara h 2.02_P P2	Ara h 2.02_P P11	Ara h 2.02_Hyp P11	Ara h 2.02_Hyp P15
1	X	X	X	X	X	X	X	X
2	-	-	-	-	-	-	-	-
3	X	-	X	X	X	-	X	X
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	X	X	X	X	X	X	X	X
7	X	X	X	X	X	X	X	X
8	X	X	X	X	X	X	X	X
9	-	-	-	X	-	-	X	X
10	X	X	X	X	X	X	X	X
11	-	-	-	-	-	-	-	-
12	X	X	X	X	X	X	X	X
13	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-
15	-	X	-	X	-	X	X	X
16	-	-	-	X	-	-	X	X
17	X	-	X	X	X	-	X	X
18	X	X	X	X	X	X	X	X
19	-	-	-	-	-	-	-	-
20	-	-	-	X	-	-	X	X
21	X	X	X	X	X	X	X	X
22	-	X	-	X	-	X	X	X
23	X	X	X	X	X	X	X	X
24	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-

27	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-	-
35	-	-	-	-	-	-	-	-

The molecular surface presentation of the four selected candidate diagnostic peptide pairs is depicted in Figure 15. Figure 15A shows the surface of Ara h 2.01 and the selected candidate diagnostic peptides, peptide 2 (cyan) and 11 (orange). In addition, Figure 15B illustrates Ara h 2.02 and the surface presentation of selected candidate peptides, peptide 2 (cyan), peptide 11 (orange) and peptide 15 (magenta). In Ara h 2.01 and Ara h 2.02 peptide 2 and peptide 11 were located on opposite protein regions. Whereas in contrast, the identified peptide pair of Ara h 2.02_Hyp (peptide 11 and 15) seemed to present two adjacent protein regions on Ara h 2.02.

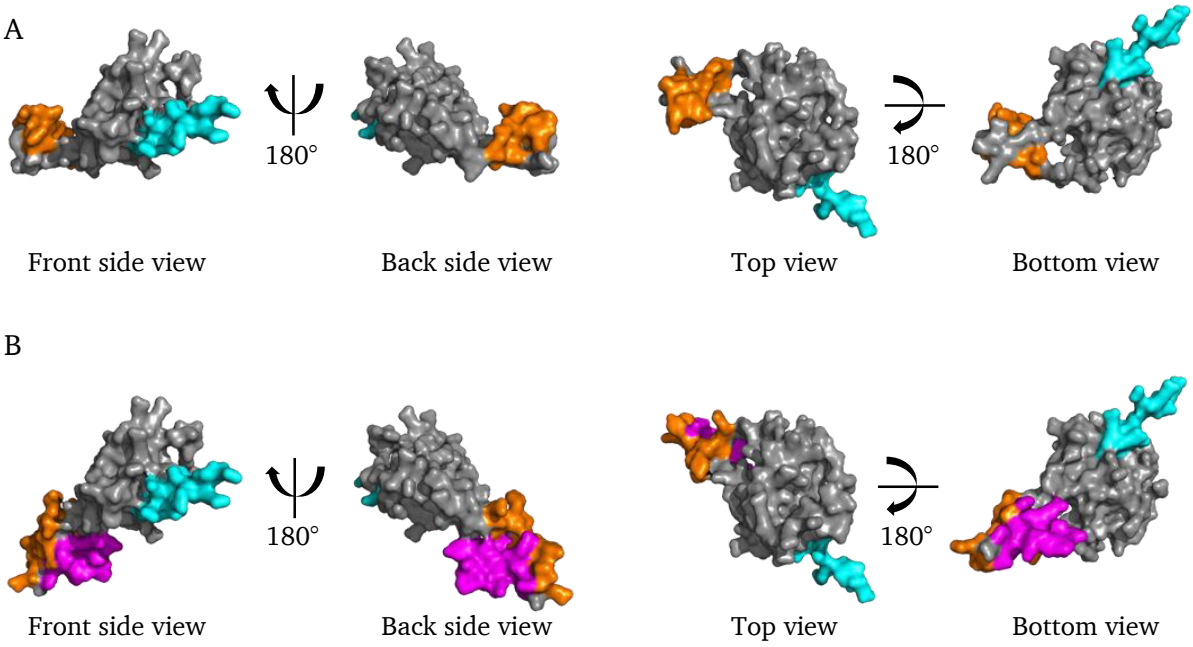


Figure 15: Surface presentation of identified candidate diagnostic peptides of Ara h 2.

Four candidate diagnostic peptide pairs could be identified of Ara h 2.01_P, Ara h 2.01_Hyp, Ara h 2.02_P and Ara h 2.02_Hyp, in total. (A) Molecular surface presentation of peptide 2 and 11 of Ara h 2.01. (B) Surface presentation of peptide 2, 11 and 15 of Ara h 2.02. Peptide 2 is shown in cyan, peptide 11 in orange and peptide 15 in magenta. Images were generated using pdb 1W2Q and PyMOL.

4.1.6 Diagnostic value of candidate diagnostic peptides in comparison to full-length peanut proteins

An ROC curve analysis was performed to compare the diagnostic value of identified candidate diagnostic peptides with that of full-length recombinant peanut allergens and peanut extract. The goal was to investigate whether peptides have a diagnostic value comparable to allergen components in the *in vitro* diagnosis of peanut allergy.

For ROC curve analysis of candidate diagnostic peptides, maximum Z-scores of all selected peptides were calculated for each individual patient. For this purpose, the Z-score values were used after subtraction of the maximum Z-score of the respective non-allergic controls. This resulted in a total of five maximum Z-scores for each patient; one maximum Z-score of Ara h 1 candidate peptides, one of Ara h 2.01_P candidate peptides, one of Ara h 2.01_Hyp candidate peptides, one of Ara h 2.02_P candidate peptides and one of Ara h 2.02_Hyp candidate peptides (Table 22).

Table 22: Maximum Z-scores of candidate diagnostic peptides of Ara h 1 and Ara h 2.

Three peptides could be identified as candidate diagnostic peptides of Ara h 1 and four peptide pairs of Ara h 2.01_P, Ara h 2.01_Hyp, Ara h 2.02_P and Ara h 2.02_Hyp, in total. For each patient, maximum Z-scores were calculated of the candidate peptides of each investigated sequence of Ara h 1 and Ara h 2. Z-scores after subtraction of the controls were used.

Patient No.	Max. Z-score Ara h 1 P17, P75, P86	Max. Z-score Ara h 2.01_P P2, P11	Max. Z-score Ara h 2.01_Hyp P2, P11	Max. Z-score Ara h 2.02_P P2, P11	Max. Z-score Ara h 2.02_Hyp P11, P15
1	10.12457809	24.885217	78.4649735	24.885217	82.91889279
2	-0.736457615	-5.612952225	-5.86766421	-3.856712921	-3.211413553
3	-1.994482129	59.08290441	105.1890394	59.08290441	108.5642069
4	6.326208737	-2.174186829	-6.529736594	-3.54256173	-3.370598332
5	-0.772796256	-5.58058368	-5.58058368	-4.016345823	-3.073446339
6	84.79687282	58.36760936	58.81588431	57.86878767	62.61335991
7	0.359229488	95.69470799	106.1378765	95.69470799	109.8149618
8	-4.268148045	61.10623904	71.14677276	61.10623904	74.63243398
9	-0.896450532	-5.912506537	4.091164073	-3.816882925	3.656131376
10	44.84097185	77.86173548	82.48197642	77.86173548	85.92152561
11	-0.92839475	-5.508709704	-5.850810739	-3.002554353	-2.147028281
12	68.73450625	68.56886998	72.7396908	42.40837528	76.22798679
13	-1.350007928	-4.467335497	-1.048484363	-2.495774503	1.642752389
14	0.151565805	-3.785217339	-4.363661212	-2.371823791	-1.255727394
15	94.7420294	57.7869737	77.71325237	38.7617689	81.38323543
16	-0.78213749	-5.37099605	16.41596601	-3.455771856	21.69132961
17	156.20918	73.68120369	73.68120369	73.68120369	76.27500298
18	81.98156277	93.44976207	105.6427058	93.44976207	109.3633373
19	-1.290067053	-5.432354349	-4.325158196	-2.159810059	-1.604273017

20	-0.65664687	-6.048067276	55.90512492	-3.611842067	23.75118361
21	35.48695418	23.16422157	22.81892271	25.5028631	26.50583576
22	2.213755576	21.04358948	81.65369551	13.36838318	85.17403264
23	76.82544973	73.82538437	75.40045852	75.01097686	79.14414255
24	-1.318952241	-4.624911449	-5.769823188	-3.370943809	-2.674381282
25	-5.134985571	-3.857155314	-3.937960277	-2.127996643	-1.111020263
26	-1.204178077	-6.493693218	-8.253576534	-5.046534243	-4.371282651
27	0.84955672	-5.067266426	-5.568557787	-3.618857988	-2.95359942
28	0.241959278	-5.496051821	-5.82621013	-3.656318789	-2.954374939
29	0.192742855	-5.125513941	-5.772635938	-3.64008616	-2.679465266
30	-0.046595008	-5.523266941	-4.352039899	-3.032464668	-2.44833489
31	1.006891245	-5.635144517	-6.455953423	-3.234564066	-3.117862372
32	-0.797950948	-5.740311195	-6.524504949	-3.484622868	-2.951030951
33	-0.360699853	-5.699112939	-6.555281448	-3.529978186	-2.850839539
34	1.836338996	-5.986133695	-6.703365933	-4.176955205	-2.726327955
35	-0.994015772	-6.05307505	-6.43796171	-4.083414897	-2.816811322

Figure 16 shows the result of the ROC curve analysis. Again, sIgE to rAra h 2.01, determined by ImmunoCAP™ analysis, served as a reference, as it had the highest diagnostic value in this study population. As already shown in Figure 5, rAra h 2.02 had, with an AUC of 0.86, so far the highest diagnostic value of investigated recombinant proteins in immunoblot analysis. In contrast, rAra h 1 had the lowest diagnostic value in immunoblot analysis. The same applied for the identified candidate diagnostic peptides of Ara h 1 that had of all identified candidate diagnostic peptides the lowest AUC (0.66) and consequently the lowest diagnostic value in this study population. The four identified candidate diagnostic peptide pairs of Ara h 2 had AUCs between 0.83 and 0.90. Of these four peptide pairs, the two peptide pairs of Ara h 2.01_Hyp and Ara h 2.02_Hyp had, with AUC 0.90 and 0.87, respectively, the highest diagnostic value, which was comparable to that of rAra h 2.02 in immunoblot analysis.

In immunoblot analysis, IgE binding to full-length rAra h 2.02 resulted in a sensitivity of 78% at a specificity of 100%. Moreover, using a Z-score of > 2 as cut-off both peptide pairs of Ara h 2.01_Hyp and Ara h 2.02_Hyp showed a sensitivity of 70% and a specificity of 100% comparable to that of rAra h 2.02 in immunoblot analysis. In comparison, ImmunoCAP™ using rAra h 2.01 reached at a sensitivity of 96% a specificity of 92% in this study population.

Consequently, three reagents with a high diagnostic value could be identified, namely rAra h 2.02 in immunoblot and both peptide pairs of Ara h 2.01_Hyp and Ara h 2.02_Hyp in multipeptide microarray analysis, which should be compared in detail with the reference rAra h 2.01 used in ImmunoCAP™ analysis.

The comparison revealed that almost all (22/23) peanut-allergic and just one tolerant patient showed a sensitization to rAra h 2.01 according to ImmunoCAP™ analysis. In immunoblot and microarray analysis, no tolerant patient showed an IgE binding to full-length rAra h 2.02 and to the two candidate diagnostic peptide pairs of Ara h 2.01_Hyp and Ara h 2.02_Hyp, respectively. Comparing ImmunoCAP™ with immunoblot revealed that rAra h 2.01 in ImmunoCAP™ was more sensitive compared to rAra h 2.02 in immunoblot (96% vs. 78%) and performed almost perfectly. Nevertheless, a positive sensitization of patient 11 could only be detected with rAra h 2.02 in the immunoblot analysis. In addition, comparing the IgE binding to rAra h 2.02 (18/23 peanut-allergic children) with the IgE binding to the two candidate diagnostic peptide pairs of Ara h 2.01_Hyp and Ara h 2.02_Hyp (16/23 peanut-allergic children, respectively) revealed that serum IgE of patients 5, 11, 19 bound only to full-length rAra h 2.02 used in immunoblot analysis. Whereas serum IgE of patient 16 bound only to peptides containing hydroxylated proline residues of Ara h 2.01_Hyp and Ara h 2.02_Hyp.

Summing up, despite the fact that different methods were compared for their diagnostic value in ROC curve analysis in this study, it could be shown that two peptide pairs of Ara h 2.01_Hyp and Ara h 2.02_Hyp could be identified that had a diagnostic value comparable to that of full-length rAra h 2.02 used in immunoblot analysis. Furthermore, it could be shown that proline hydroxylation had a positive impact on the diagnostic sensitivity and accuracy. These results should be taken into account in further investigations.

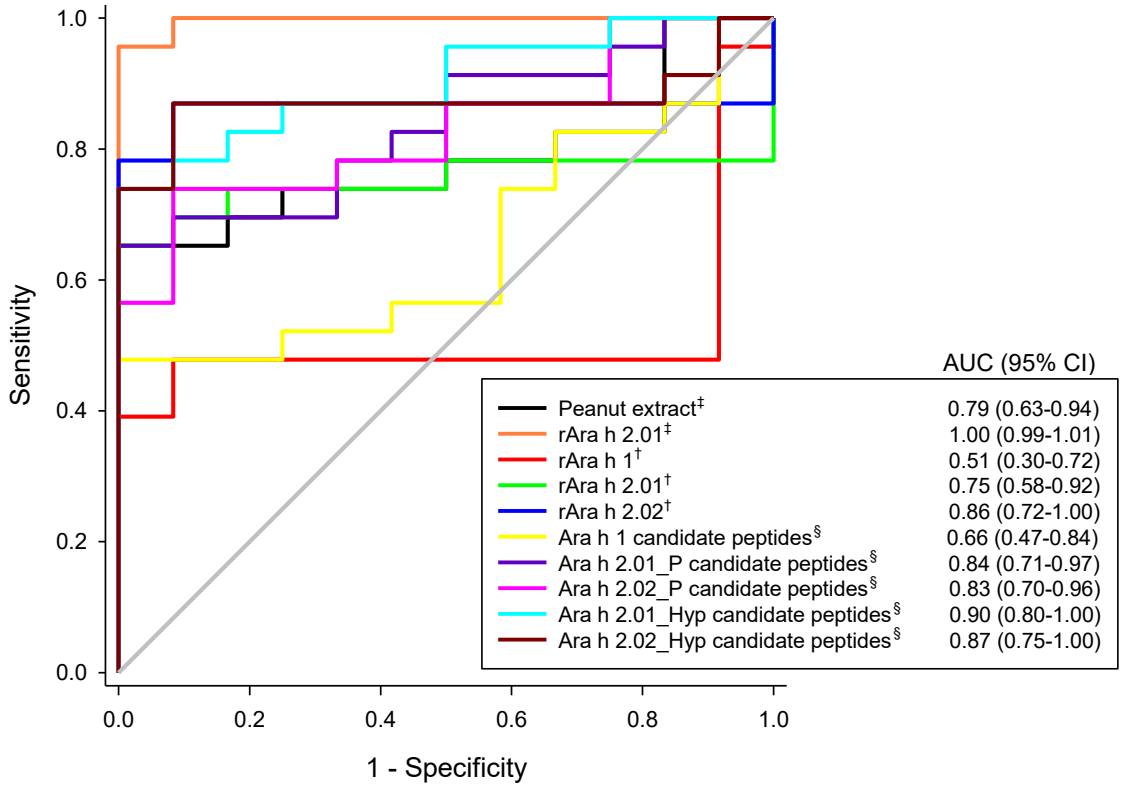


Figure 16: Receiver operating characteristic (ROC) curve analysis of sIgE to peanut extract, full-length recombinant proteins and identified candidate diagnostic peptides.

ROC curves of sIgE to peanut extract (black), rAra h 2.01 (orange) determined by ImmunoCAP™ analysis (‡) and to rAra h 1 (red), rAra h 2.01 (green) and rAra h 2.02 (blue) quantified by immunoblot analysis (†). Performance of identified candidate diagnostic peptides of Ara h 1, Ara h 2.01_P, Ara h 2.01_Hyp, Ara h 2.02_P and Ara h 2.02_Hyp are depicted in yellow, purple, pink, cyan and brown, respectively. For ROC curve analysis, ImmunoCAP™ sIgE values > 100 kU_A/L were set to 100 kU_A/L and the sIgE levels quantified by means of immunoblot analysis were subtracted by two times the respective sIgE level of the non-allergic control serum. For ROC curve analysis of candidate diagnostic peptides, calculated maximum Z-scores were used. Z-scores were determined by microarray analysis (§). The gray diagonal line (AUC 0.5) represents a test without discriminatory ability. AUC, area under the ROC curve; CI, confidence interval.

Finally, as the number of some candidate diagnostic peptides could be further narrowed while maintaining the specificity and sensitivity at a cut-off of Z-score > 2, the influence of narrowing on the respective AUC was investigated. Peptide 2 and peptide 11 of Ara h 2.01_Hyp reached together a sensitivity of 70% and a specificity of 100%. Peptide 11 alone showed the same sensitivity and specificity pair and revealed an AUC of 0.89, which was comparable to that of both initially identified candidate diagnostic peptides of Ara h 2.01_Hyp (AUC 0.90). The same applied for peptide 15 of Ara h 2.02_Hyp, where ROC curve analysis revealed an AUC of 0.86, which was also comparable to that of both selected candidate diagnostic peptides (AUC 0.87).

In contrast using solely peptide 11 of Ara h 2.02_Hyp a slightly lower AUC (0.84) was obtained (data not shown).

4.1.7 Relevance of linear and conformational IgE epitopes

For the development of safe hypoallergenic immunotherapeutic approaches, it is important to know the contribution of linear and conformational IgE epitopes. Therefore, whole peanut extract was reduced and alkylated and Ara h 2, the major allergen in this study population, was analyzed for its IgE-binding capacity in comparison to native Ara h 2 (Figure 18).

Mass spectrometry analysis confirmed the identity of Ara h 2 and Ara h 6 in both extracts (Figure 17, Table 23). In the native peanut extract (lane 1 Figure 17) three bands were analyzed. MS analysis identified unequivocally Ara h 2.0201 and Ara h 2.0101 in bands 1 and 2, respectively. Moreover, in band 2 peptides belonging to Ara h 2.0201 and Ara h 6 were additionally identified. Band 3 of the native peanut extract contained Ara h 2.0201 as well as Ara h 6. In the reduced/alkylated peanut extract (lane 2 Figure 17) MS analysis identified in band 4, 5 and 6, which correspond to band 1, 2 and 3 of the native peanut extract, Ara h 2.0201, Ara h 2.0101 and Ara h 6, respectively.

However, as in bands 3, 4 and 5 only C-terminal peptides were detected, the presence of the variants Ara h 2.0102 and Ara h 2.0202 described by Hales *et al.* in 2004 cannot be confirmed or excluded (Hales et al. 2004). Sequence alignment of the Ara h 2 sequences described by Hales *et al.* and identified isoform-specific peptides are shown in the appendix (Figure A22 and Table A52). The shown MS data only allowed the conclusion that either Ara h 2.0201 or Ara h 2.0102 is present. The same applied for Ara h 2.0101 or Ara h 2.0202.

On the other hand, the reported Ara h 2 sequences by Hales and co-workers were not proven to exist at the protein level and are not registered by the IUIS allergen nomenclature sub-committee. Accordingly, we strictly referred throughout the entire study to the IUIS database entry Ara h 2.0101 and Ara h 2.0201 even though Ara h 2.0102 and Ara h 2.0202 would in many cases match our data as well.

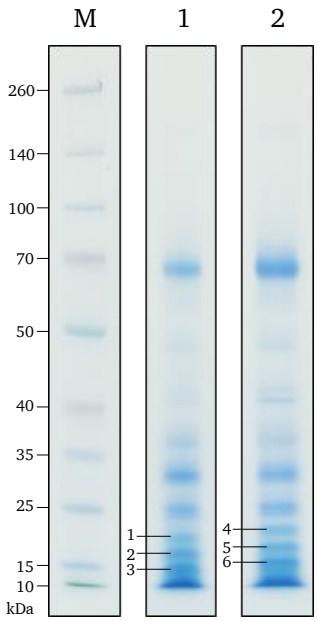


Figure 17: SDS-PAGE of native and reduced/alkylated peanut extract for MS analysis.

Lane 1, native peanut extract; lane 2, reduced/alkylated peanut extract. Samples were analyzed under reducing conditions. Bands 1-6 were analyzed by Mass spectrometry. M, Spectra™ Multicolor Broad Range Protein Ladder.

Table 23: Identification of Ara h 2 and Ara h 6 in peanut extract.

Band	Acc.No. GB	Acc. No. UP	Description	S	P	C	E
1	AAN77576	Q6PSU2*	Ara h 2.0201 Conglutin-7 *Arachis hypogaea*	6588	12	47.1	6.4
2	AAK96887	Q6PSU2*	Ara h 2.0101 Conglutin-7 *Arachis hypogaea*	12146	14	73.2	6.6
2	AAN77576	Q6PSU2*	Ara h 2.0201 Conglutin-7 *Arachis hypogaea*	10006	13	50.0	6.9
2	AAD56337	Q647G9	Ara h 6.0101 Conglutin *Arachis hypogaea*	2095	9	46.9	3.7
3	AAN77576	Q6PSU2*	Ara h 2.0201 Conglutin-7 *Arachis hypogaea*	3879	7	28.5	9.1
3	AAD56337	Q647G9	Ara h 6.0101 Conglutin *Arachis hypogaea*	14300	15	58.0	4.7
4	AAN77576	Q6PSU2*	Ara h 2.0201 Conglutin-7 *Arachis hypogaea*	3924	10	36.0	4.1
5	AAK96887	Q6PSU2*	Ara h 2.0101 Conglutin-7 *Arachis hypogaea*	6404	10	47.0	5.0
6	AAD56337	Q647G9	Ara h 6.0101 Conglutin *Arachis hypogaea*	439	4	24.1	4.8

Acc.No. GB, accession number of GenBank; Acc.No. UP, accession number of Uniprot; *isoforms were collapsed into one UniProt entry only; S, PLGS protein score; P, number of identified peptides; C, protein sequence coverage %; E, precursor RMS mass error [ppm].

Nevertheless, based on their reported molecular weights and published data from Chen *et al.* it was assumed that band 1 and 4 contained Ara h 2.02 (~19 kDa), band 2 and 5 Ara h 2.01

(~17 kDa) and band 3 and 6 Ara h 6 (~15 kDa) (Chen et al. 2013). Thus, in the following immunoblot shown in Figure 18 the upper, the middle and the lower of the three bands in both extracts will be referred to as Ara h 2.02, Ara h 2.01 and Ara h 6, respectively.

According to Figure 18, peanut-allergic children showed a strong IgE binding to native peanut extract, which was especially pronounced in the area of the 2S albumins Ara h 2.01 and Ara h 2.02.

Moreover, IgE immunoblot analysis of native (n) and reduced and alkylated (r/a) peanut extract showed that serum IgE of 16/23 (70%) peanut-allergic children (patient 1, 3, 6, 7, 8, 10, 12, 15, 16, 17, 18, 19, 20, 21, 22 and 23) still showed IgE binding to Ara h 2.02 and Ara h 2.01 after reduction and alkylation. Of these 16 children, twelve children (patient 3, 6, 7, 8, 10, 12, 15, 17, 18, 21, 22 and 23) showed significant IgE binding to r/a Ara h 2.01 and r/a Ara h 2.02. Patients 3 and 8 did not even show a change in IgE binding after reduction and alkylation. Serum IgE of the remaining four allergic patients showed a reduced IgE-binding capacity. In 5/23 (22%) peanut-allergic children (patients 2, 4, 5, 9 and 13) and in peanut-tolerant child 25 reduction and alkylation resulted in a complete loss of IgE binding to both Ara h 2 isoforms. For patients 11 and 14 hardly any IgE binding to peanut extract could be observed at all, comparable to the majority of peanut-tolerant children. In addition, as Ara h 1 is not stabilized by disulfide bonds, reduction and alkylation did not affect its secondary structure (van der Kleij et al. 2019) and thus had no impact on its IgE-binding capacity (Figure 18, band ~70 kDa).

However, reduction and alkylation of Ara h 6 resulted in all children (allergic and tolerant) in a complete or major loss of IgE binding.

In general, IgE binding to r/a Ara h 2 was in good agreement with the detected IgE binding to Ara h 2-derived peptides. Serum IgE of peanut-tolerant children bound neither to any Ara h 2-derived peptide nor to r/a Ara h 2. Except for peanut-allergic patients 9 and 19, IgE binding to r/a Ara h 2 was in complete accordance with the microarray analysis. Patient 9 recognized few Ara h 2-derived peptides, however showed hardly any IgE binding to r/a Ara h 2. For patient 19 the opposite could be observed. This patient showed a weak IgE binding to r/a Ara h 2, but no binding to Ara h 2-derived peptides.

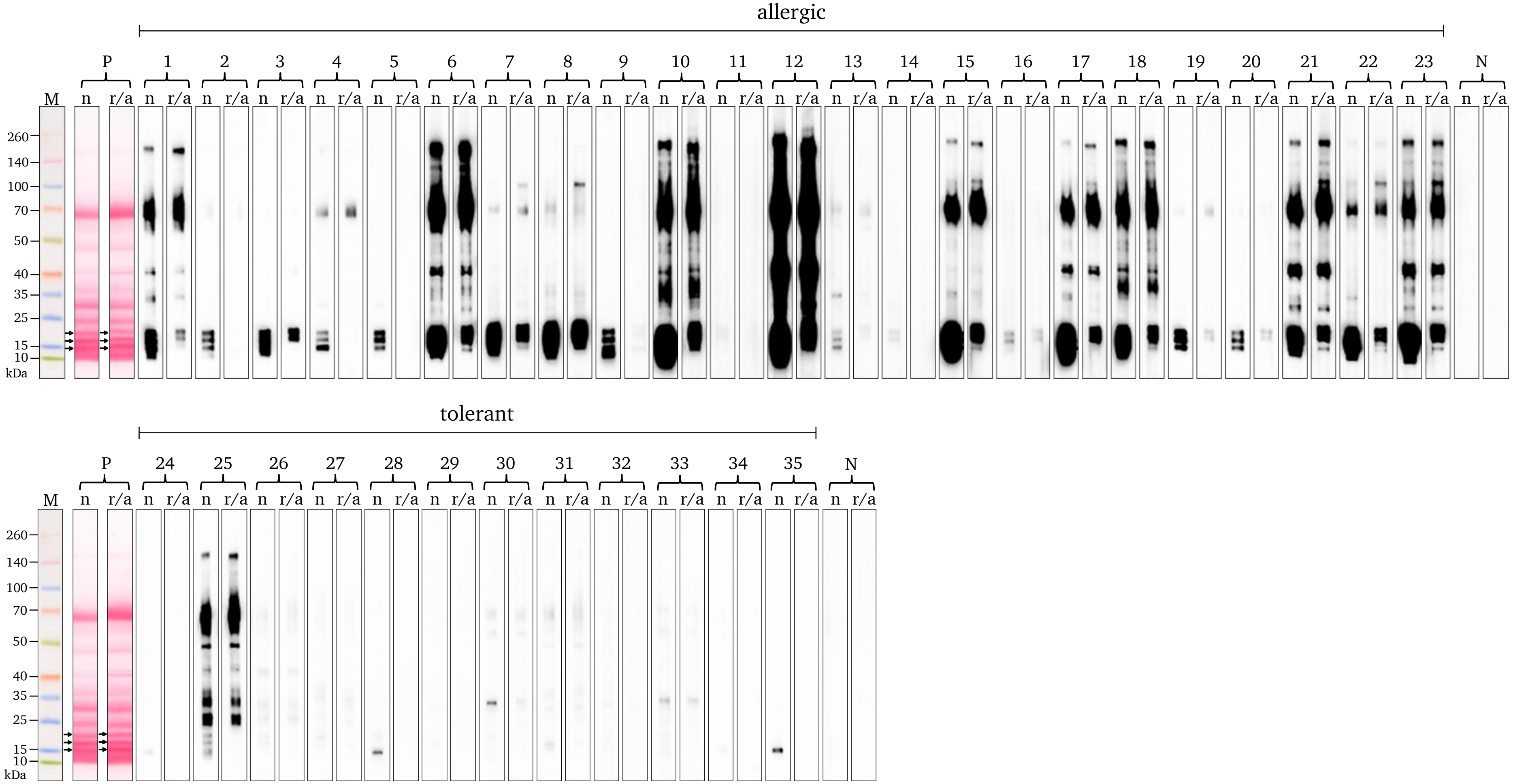


Figure 18: Serum IgE binding to native and reduced/alkylated peanut extract.

IgE binding of sera 1-35 (Table 14) to native (n) and reduced and alkylated (r/a) peanut extract. Peanut extracts were analyzed under reducing conditions. The detection after 1 min of exposure is shown. Arrows indicate Ara h 2.02 (~19 kDa), Ara h 2.01 (~17 kDa) and Ara h 6 (~15 kDa). M, Spectra™ Multicolor Broad Range Protein Ladder; P, total protein stained with Ponceau S; N, non-allergic control serum.

4.1.8 Biological IgE reactivity of conformational and linear IgE epitopes

To determine the biological IgE reactivity of linear IgE-binding epitopes of Ara h 2, a mediator release assay was performed. The mediator release assay should give additional knowledge about the influence of proline hydroxylation on the allergenicity of linear IgE epitopes of Ara h 2. Therefore, rAra h 2.02 not undergoing proline hydroxylation was reduced and alkylated and compared for its allergenicity with two linear DPYSPS-containing 27-mer peptides. One of the two 27-mer peptides contained hydroxylated proline residues (27-mer peptide P^{OH}), the second only proline residues (27-mer peptide P). The loss of the secondary structure of r/a rAra h 2.02 was confirmed by CD spectroscopy (see appendix Figure A24). A minimum at ~200 nm instead of the typical α-helical two minima (208 nm and 222 nm) as shown in Figure 2 for properly folded rAra h 2.01 and rAra h 2.02, revealed the unfolding of r/a rAra h 2.02. In addition, both 27-mer peptides presented no secondary structure elements (appendix Figure A24). Moreover, as expected, reduction and alkylation of rAra h 1 had no influence on its secondary structure. The CD spectrum of r/a rAra h 1 (appendix Figure A24) was almost identical to untreated rAra h 1 shown in Figure 2.

After confirming an unfolded state of r/a rAra h 2.02 and the absence of secondary structure elements in 27-mer peptides, three serum pools composed of peanut-allergic patients with similar IgE-binding characteristics were generated. Serum pool 1 was composed of peanut patients 3 and 17. Both allergic patients showed a serum IgE binding to rAra h 2 and nAra h 2 as well as to r/a nAra h 2. In addition, in these two patients proline hydroxylation was necessary to induce positive serum IgE binding to peptides containing the DPYSPS motif (peptides 9-14 of Ara h 2.01_Hyp and peptides 9-17 of Ara h 2.02_Hyp). Furthermore, both patients recognized peptide 2 and patient 17 additionally recognized peptide 3. Serum pool 2 included patients 6-8, 10, 12, 15, 18, 21-23. These patients showed, in agreement with patients of pool 1, serum IgE binding to rAra h 2, nAra h 2 and r/a nAra h 2. However, in contrast to serum pool 1, patients of pool 2 recognized the DPYSPS motif with and without hydroxyproline residues. Furthermore, the great majority of patients of pool 2 recognized peptide 2 (8/10 patients) and 6/10 patients recognized other Ara h 2-derived peptides. In contrast to both serum pools 1 and 2, serum pool 3 was composed of patients showing no IgE binding to Ara h 2-derived peptides. Patients of serum pool 3 (patient 2, 4, 5, 13 and 19) showed no or weak serum IgE binding to rAra h 2 (with the exception of patient 19). Furthermore, these patients recognized nAra h 2, whereas reduction and alkylation of peanut extract resulted in a loss or at least a strong reduction in the serum IgE binding to r/a nAra h 2 in these patients.

The mediator release of RBL cells sensitized with serum pools 1-3 is shown in Figure 19. As negative control, cells were additionally sensitized with non-allergic control serum N. None of

the tested molecules triggered mast cell degranulation from cells sensitized with control serum N (data not shown).

As expected, cells sensitized with serum pool 3 showed only a mediator release when stimulated with native/untreated peanut extract and properly folded rAra h 2.02 (Figure 19C). For these patients proper folding of Ara h 2 is mandatory to induce mast cell degranulation demonstrating that these patients are sensitized to conformational epitopes on Ara h 2. In addition, the lack of a mediator release when cells were stimulated with r/a peanut extract or rAra h 1 showed that the whole IgE reactivity/allergenicity of peanut extract was caused by conformational IgE-epitopes of Ara h 2 (and potentially of Ara h 6) in these patients.

In contrast, when RBL cells were sensitized with serum pool 2 it could be shown that linear IgE epitopes of Ara h 2 were able to induce a mast cell degranulation (Figure 19B). The linear 27-mer peptide containing hydroxylated proline residues (27-mer peptide P^{OH}) could induce a maximum mediator release of ~70%, which was comparable to the maximum release induced by rAra h 2.02. In contrast, the 27-mer peptide without hydroxylated proline residues (27-mer peptide P) and r/a rAra h 2.02 did not show any or at least a strongly reduced allergenic activity. The 27-mer peptide P^{OH} displaying linear IgE-epitopes showed a potency comparable to rAra h 2.02 displaying conformational IgE epitopes. However, considering the molar ratio of both (~1:5), both mediator release curves are separated by a factor of ~10 showing that, at the molar level, rAra h 2.02 was tenfold more IgE reactive than the 27-mer peptide P^{OH}. rAra h 2.02 showed, compared to rAra h 1, a 100 times higher allergenic activity. Comparable to the unaffected IgE-binding capacity of Ara h 1 shown in Figure 18, reduction and alkylation also had no impact on the allergenicity of rAra h 1 (Figure 19B and 19C).

Due to low serum availability, cells sensitized with serum pool 1 could not be investigated with all antigens (Figure 19A). Based on the illustrated curves it can be seen that, although rAra h 2.02 was ~1000 times more IgE reactive, the 27-mer peptide P^{OH} can still trigger a mast cell degranulation. Comparable to serum pool 2, the 27-mer peptide P could not induce a mediator release. r/a rAra h 2.02 did not show any allergenic activity in serum pool 1, whereas in serum pool 2 a very weak mediator release could be detected, caused by additional linear IgE epitopes on Ara h 2.

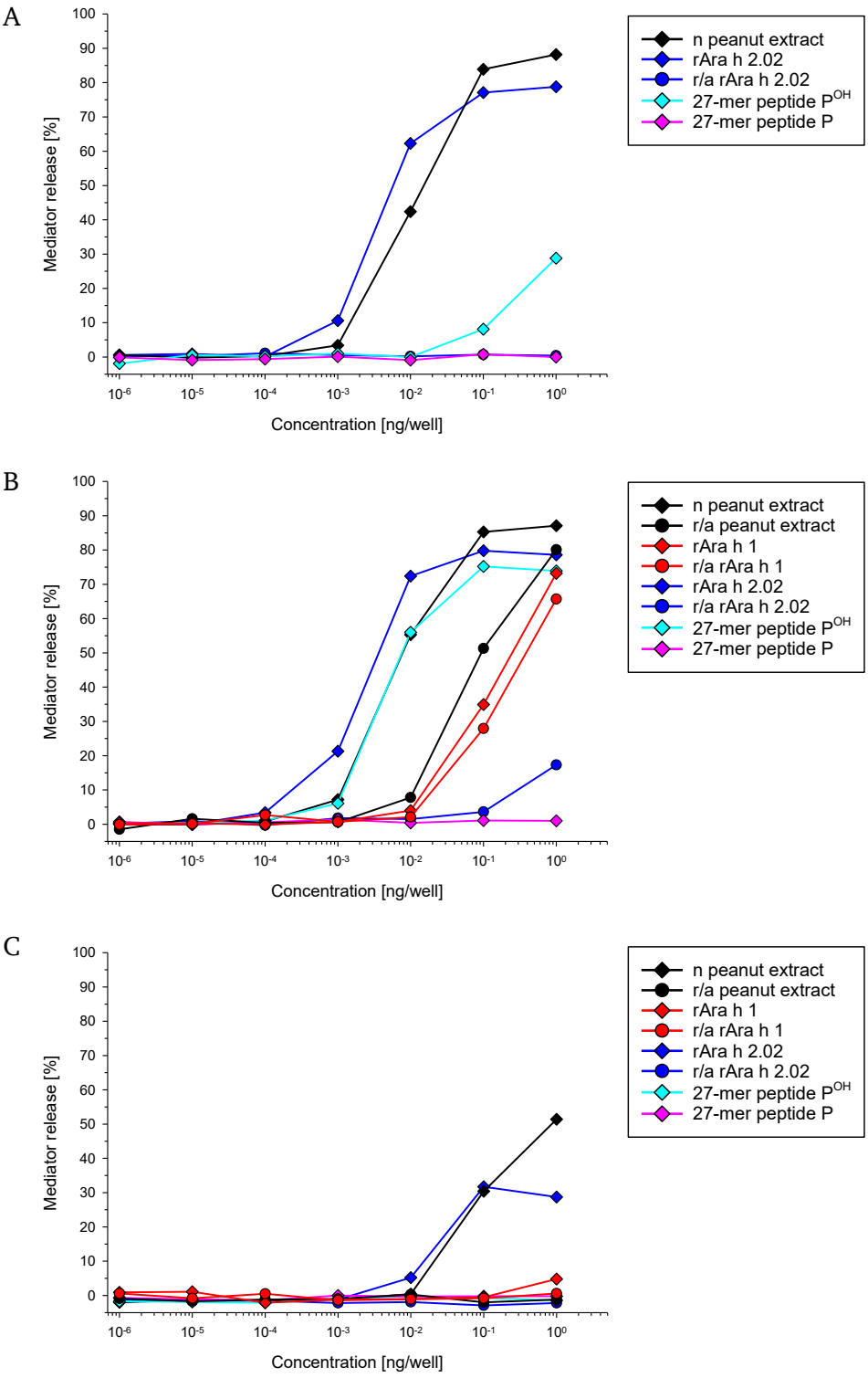


Figure 19: Mediator release induced by peanut extract, recombinant peanut allergens and 27-mer peptides. Rat basophilic leukemia cells were sensitized with either serum pool 1 (A), serum pool 2 (B) or serum pool 3 (C) and the mediator release induced by native peanut extract (black line, diamond), r/a peanut extract (black line, circle), rAra h 1 (red line, diamond), r/a rAra h 1 (red line, circle), rAra h 2.02 (blue line, diamond), r/a rAra h 2.02 (blue line, circle), 27-mer peptide P^{OH} (cyan line, diamond) and 27-mer peptide P (pink line, diamond) was determined. Serum pool 1 was composed of peanut patients 3 and 17, pool 2 of patients 6-8, 10, 12, 15, 18, 21-23 and pool 3 of patients 2, 4, 5, 13 and 19.

4.2 Pea project

4.2.1 Study population

In total, sera from nineteen children were included in this project based on a pea-specific IgE level of ≥ 0.35 kU_A/L. Patients characteristics are summarized in Table 24. Fourteen children were allergic to pea (patients 1-14) and five children (patients 15-19) were sensitized but clinically tolerant. Clinically relevant pea allergy was diagnosed according to a convincing history (10/14 pea-allergic children) or by oral food challenge (5/14 pea-allergic children). For patient 4, clinical evidence of pea allergy was given by history and food challenge. Pea allergy was excluded in 3/5 tolerant children according to a convincing history and in 2/5 children by oral food challenge. Pea related allergy symptoms ranged from mild (grade I) to severe (grade IV). Both pea study groups were composed of young children. The median age of pea-allergic children was 4 years (range 1-15 years) and of tolerant children 7 years (range 2-12 years). Pea-specific IgE ranged from 0.94 to 92.30 kU_A/L in pea-allergic children (median 6.02 kU_A/L) and from 0.40 to 3.96 kU_A/L in pea-tolerant children (median 1.27 kU_A/L).

Table 24: Clinical characteristics of the pea study population.

Patients allergic to pea (patients 1-14) and patients sensitized to pea without clinical symptoms (patients 15-19).

Patient No.	Age (y)*/sex	Clinical evidence	Symptoms to pea	Severity grading[#]	Total IgE (kU_A/L)[‡]	Pea sIgE (kU_A/L)[‡]
1	3/m	History	F, PU	I	2239.0	92.30
2	15/m	History	Em, N	II	n.d.	5.50
3	1/f	History	U, AE	II	104.0	32.10
4	2/m	History/OFC	F/GU	I/II	226.0	54.70
5	3/m	OFC	GU	II	n.d.	1.95
6	12/f	History	AE, Em	II	n.d.	1.52
7	2/m	History	AE	II	n.d.	1.21
8	4/m	DBPCFC	PU, P, R	III	n.d.	45.30
9	1/f	OFC	F, C, R	III	n.d.	0.94
10	6/m	OFC	PU, R, IT	III	n.d.	8.89
11	6/m	History	Em, GU, W, D	IV	141.0	23.10
12	7/f	History	N	II	459.0	5.14
13	4/m	History	AE	II	n.d.	2.12
14	2/m	History	Em, SA	II	2748.0	6.53
15	2/m	History	none	-	2813.0	1.27
16	12/m	OFC	none	-	n.d.	3.96
17	7/m	DBPCFC	none	-	152.0	1.02
18	2/m	History	none	-	64.6	0.40
19	8/m	History	none	-	7663.0	3.59

*Age at time of blood sampling; f, female; m, male; n.d., not determined; DBPCFC, double-blind placebo-controlled food challenge; OFC, open food challenge; AE, angioedema; C, conjunctivitis; D, dyspnea; Em, emesis; F, flush; GU, generalized urticaria; IT, itching throat; N, nausea; P, pruritus; PU, perioral urticaria; R, rhinitis; SA, stomach ache; U, urticaria; W, wheezing; [#]severity grading according to the grading system developed by Sampson (Sampson 2003); [‡] determined by ImmunoCAP™.

4.2.2 Generation and physicochemical characterization of rPis s 1, rPA1 and rPA2

Comparable to the peanut project, cDNA sequences encoding rPis s 1, rPA1 and rPA2 (appendix Figure A6, A8 & A9) could be successfully cloned into pPICZαA and afterwards integrated into *P. pastoris* genome. Recombinant PA1 and PA2 were successfully expressed using *Pichia pastoris* without any optimization strategies. However, the expression of full-length rPis s 1 in *Pichia pastoris* failed and resulted in two truncated proteolytic products (appendix Figure A12). MS analysis identified the ~20 kDa and the ~27 kDa proteolytic products as N- and C-terminal fragments of rPis s 1, respectively (data not shown). In addition, a potential cleavage site at K168 and R187 (referring to the UniProt protein sequence) could be identified by MS analysis. However, despite several optimization strategies such as the addition of protease inhibitors or the use of *P. pastoris* SMD1168H protease-deficient strain, rPis s 1 could not be expressed as full-length protein in *Pichia pastoris*. Consequently, rPis s 1 was finally expressed in *Escherichia coli*. Recombinant PA1 was expressed as proprotein composed of two chains linked by a propeptide (6 amino acid residues). Furthermore, rPA1 contained an additional amino-terminal propeptide (8 amino acid residues). For sequence information of rPA1 see Figure A8 in the appendix.

rPA1 and rPA2 were secreted into *Pichia pastoris* cell culture supernatant and rPis s 1 was expressed in *E. coli* inclusion bodies. The three pea proteins were subsequently purified via their His-tag using IMAC. After IMAC, rPA1 and rPA2 were further purified by SEC. Compared to the 7S globulin rPis s 1 that needed a high salt concentration (500 mM NaCl) to stay soluble, the pea albumins PA1 and PA2 could be stored at low salt concentrations (10 mM NaCl). After purification, mass spectrometry analysis confirmed the identity of rPis s 1, rPA1 and rPA2. The determined sequence coverages for rPis s 1, rPA1 and rPA2 were 83.1%, 18.4% and 83.8%, respectively (Table 25).

Table 25: Identity confirmation of recombinant pea proteins by mass spectrometry analysis.

Protein	Accession number[#]	PLGS protein score	Number of identified peptides	Sequence coverage	Mass error*
rPis s 1	PEI112	26020	51	83.1%	5.9
rPA1	PEI081	1683	2	18.4%	8.8
rPA2	PEI080	13291	32	83.8%	4.4

[#]PEI, internal accession number; *precursor RMS mass error [ppm].

The purity of the three recombinant pea proteins was analyzed on Coomassie-stained SDS-PAGE (Figure 20A). As shown in Figure 20A lane 1-3, IMAC with or without subsequent SEC resulted in highly pure recombinant protein preparations. Secondary structure integrity of recombinant

pea proteins was analyzed by CD spectroscopy (Figure 20B). The CD spectrum of rPis s 1 indicated predominant β-sheet conformation as a minimum at~215 nm and a maximum at ~198 nm could be detected. In contrast, the CD spectrum of rPA1 had two minima at approximately 208 and 222 nm and a maximum at 197 nm. This CD spectrum was comparable to the CD spectra of both Ara h 2 isoforms (Figure 2) and was indicative for an α-helical protein. While a native-type secondary structure could be detected for rPis s 1 and rPA1, the CD spectrum of rPA2 showed a high content of unstructured protein. Moreover, using dynamic light scattering hydrodynamic radii of 5.1 ± 0.7 nm, 3 ± 0.7 nm and 14.8 ± 1.6 nm could be determined for rPis s 1, rPA1 and rPA2, respectively (inset Figure 20B). The determined hydrodynamic radii of rPis s 1 and rPA1 indicated a possibly trimeric protein and a monomeric protein in solution, respectively. In contrast, the R_H of rPA2 indicated a strong tendency to oligomerization.

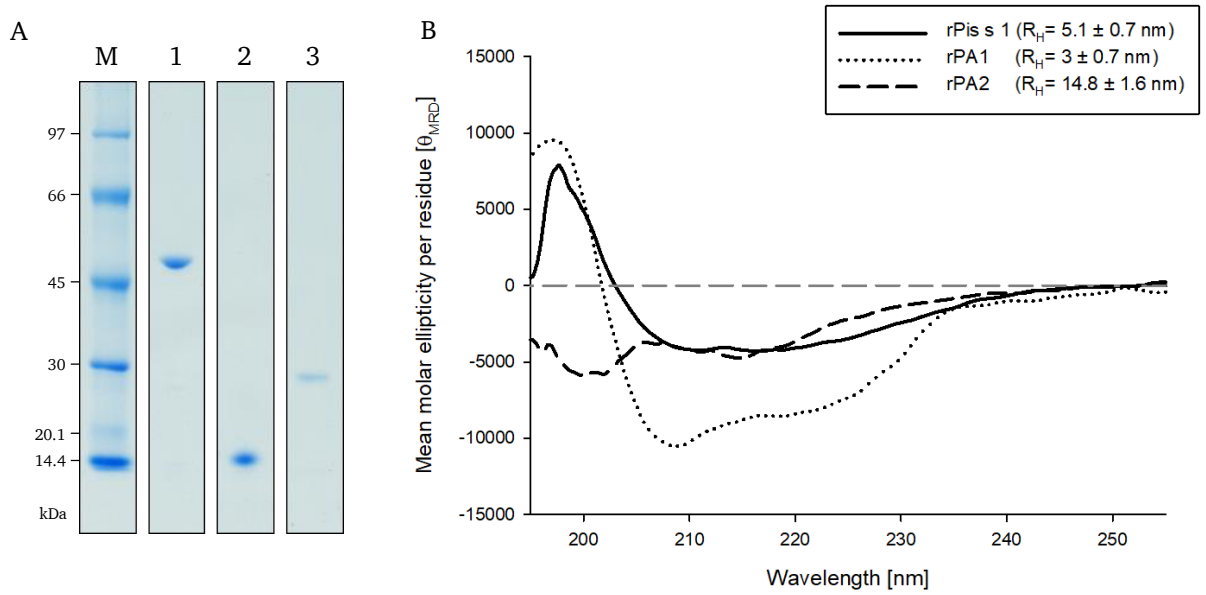


Figure 20: Purity and physicochemical characterization of rPis s 1, rPA1 and rPA2.

(A) Coomassie-stained SDS-PAGE of rPis s 1 (lane 1; 2 μg/cm), rPA1 (lane 2; 2 μg/cm) and rPA2 (lane 3; 4 μg/cm). Protein samples were analyzed under reducing conditions. M, low-molecular weight marker. (B) Far-UV CD-spectra (195-255 nm) of rPis s 1 1 (solid line), rPA1 (dotted line) and rPA2 (dashed line). Samples were measured at 4 μM, 11 μM and 6 μM, respectively. The inset depicts the hydrodynamic radius (R_H) ± SD.

4.2.3 Relevance of Pis s 1, PA1 and PA2

As currently serological diagnosis of pea allergy is solely based on pea total protein extract due to a lack of single pea allergen components, the relevance of Pis s 1, PA1 and PA2 was investigated. Therefore, IgE immunoblot and IgE immunoblot inhibition analyses as well as a mediator release assay were performed.

Initially, pea-allergic and sensitized but tolerant children were analyzed for their serum IgE binding to rPis s 1, rPA1 and rPA2 in immunoblot analysis (Figure 21). Due to no significant sequence similarity, rPA1 and rPA2 were analyzed simultaneously for their IgE binding in one lane (Figure 21B).

Compared to pea-tolerant children, allergic children showed strong IgE binding to rPis s 1. In addition, serum IgE binding to degradation products of rPis s 1 could also be observed (Figure 21A, open triangle). In contrast, with the exception of patient 1, neither allergic nor tolerant patients showed IgE binding to rPA1 and rPA2.

Consequently, as relevant IgE binding could only be detected to rPis s 1 (Figure 21A), compared to rPA1 and rPA2 (Figure 21B), only rPis s 1 sIgE levels were densitometrically quantified.

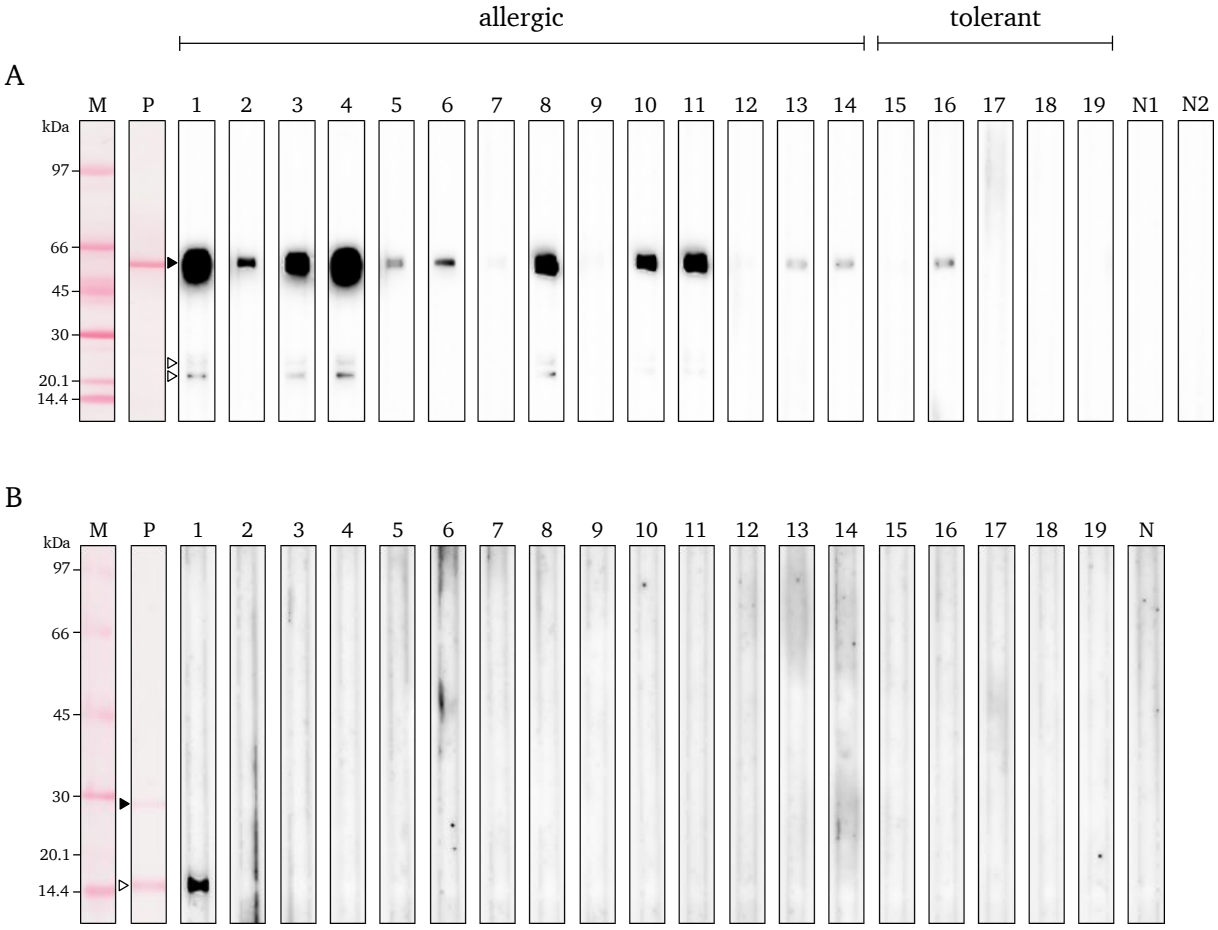


Figure 21: Serum IgE binding of pea-allergic and sensitized but tolerant children to rPis s 1, rPA1 and rPA2.
IgE binding of sera from pea-allergic children (lane 1-14, patients' sera 1-14 according to Table 24) and tolerant children (lane 15-19, patients' sera 15-19 according to Table 24) to rPis s 1 (A) and to rPA1 and rPA2 (B) after 30 sec and 5 min exposure, respectively. Protein samples were analyzed under reducing conditions. (A) filled triangle, full-length rPis s 1; open triangle, degradation products of rPis s 1. (B) filled triangle, rPA2; open triangle, rPA1. M, low-molecular weight marker; P, total protein stained with Ponceau S; N, non-allergic control serum; N1 or N2, non-allergic control serum on membrane 1 or 2.

As the commercially available ImmunoCAP™ does not contain Pis s 1, the standard for quantifying rPis s 1 sIgE was missing in this project. Therefore, a different strategy for the quantification of the rPis s 1 sIgE level was developed based on the IgE immunoblot inhibition shown in Figure 22. In this immunoblot, IgE binding to pea total protein extract was inhibited by the addition of rPis s1.

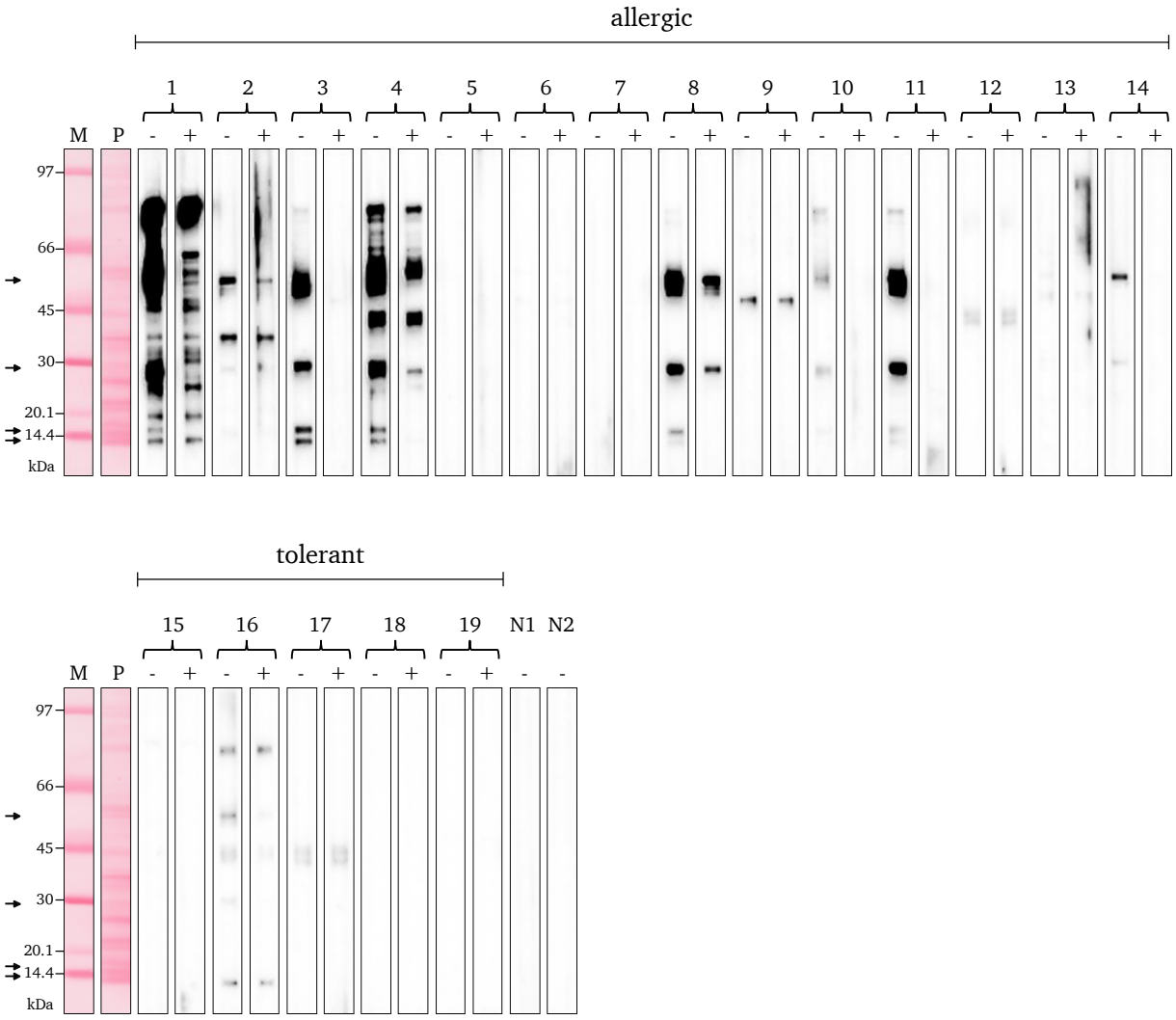


Figure 22: IgE immunoblot inhibition using rPis s 1.

IgE immunoblot of sera from pea-allergic children (patients 1-14) and pea-tolerant children (patients 15-19) to pea extract. Pea extract was analyzed under reducing conditions. The detection after 1 min of exposure is shown. Serum samples were preincubated with rPis s 1 (+) or untreated (-). Arrows illustrate full-length Pis s 1 and its proteolytic subunits (< 30 kDa). M, low-molecular weight marker; P, total protein stained with Ponceau S; N1 and N2; non-allergic control serum (N) used on membrane 1 and 2, respectively.

Using densitometric quantification relative to uninhibited pea total protein, percent inhibition was calculated for each patient (Table 26, column 2). The inhibition analysis will be discussed in detail later, first the quantification of rPis s 1 sIgE will be described in more detail.

As shown in Figure 22 and Table 26 (column 2), rPis s 1 completely inhibited IgE binding to pea total protein in patients 3, 11 and 14. Accordingly, IgE binding to pea total protein was merely related to Pis s 1, and sIgE to pea extract could be considered identical to sIgE to rPis s 1 in these patients. Using these three assumed rPis s 1 sIgE values (32.10, 23.10 and 6.5 kU_A/L) and the corresponding densitometric signal intensities of the immunoblot (Figure 21A), a linear regression was performed and the resulting calibration line was used to determine the rPis s 1 sIgE levels of all sera. Resulting rPis s 1 sIgE levels of pea-allergic and tolerant children and of the non-allergic control serum N used on both membranes (N1 and N2) are shown in Table 26 (column 3).

Table 26: Percent inhibition of IgE binding to pea total protein by rPis s 1, quantified rPis s 1 sIgE levels and pea sIgE levels of pea-allergic and tolerant children.

N1 and N2, non-allergic control serum on membrane 1 and 2, respectively. Patients 1-7 were analyzed for their IgE binding to rPis s 1 on membrane 1 and patients 8-19 on membrane 2.

Patient No.	% IgE inhibition of pea extract by rPis s 1[†]	rPis s 1 sIgE (kU_A/L)[†]	Pea sIgE (kU_A/L)[‡]
1	39.07	42.740	92.30
2	19.08	11.797	5.50
3	96.55	29.788	32.10
4	60.59	45.588	54.70
5	n.d.	8.001	1.95
6	n.d.	8.426	1.52
7	n.d.	(5.149)	1.21
8	57.91	23.659	45.30
9	26.07	(4.985)	0.94
10	81.62	16.115	8.89
11	98.17	22.518	23.10
12	0	(5.567)	5.14
13	n.d.	6.249	2.12
14	99.93	6.146	6.53
15	n.d.	(5.155)	1.27
16	65.61	6.879	3.96
17	0	(5.556)	1.02
18	n.d.	(5.107)	0.40
19	n.d.	(5.197)	3.59
N1	n.d.	4.857	n.d.
N2	n.d.	4.914	n.d.

[‡]determined by ImmunoCAP™; [†]determined by immunoblot analysis; n.d., not determined; () sIgE levels in parenthesis are below method cut-off (LOD).

As in the peanut project, serum IgE binding in immunoblot (Figure 21) was considered positive when the quantified specific IgE level (kU_A/L) exceeded the respective calculated limit of detection (LOD).

Using two times the densitometric signal intensity of the non-allergic control serum N inserted into the linear regression equation and resolved for sIgE, the LOD was calculated. This resulted in a LOD1 of 5.889 kU_A/L for patients 1-7 and in a LOD2 of 6.005 kU_A/L for patients 8-19. Considering the respective LOD, serum IgE of 11/14 (79%) pea-allergic children and of 1/5 (20%) pea-tolerant children bound to rPis s 1. In pea-allergic children, rPis s 1 sIgE levels (> LOD) were elevated ranging from 6.15 to 45.59 kU_A/L (median 16.12 kU_A/L). In contrast, only tolerant patient 16 displayed sIgE binding to rPis s 1 of 6.88 kU_A/L.

The elevated rPis s 1 sIgE levels in pea-allergic patients correlated well with the elevated pea extract sIgE levels. As shown in Figure 23, a linear correlation (R^2=0.8171) between sIgE to pea extract and sIgE to rPis s 1 (> LOQ) was observed. Depending on the respective immunoblot membrane, LOQ's of 7.954 kU_A/L (LOQ1 for patients 1-7) and of 8.185 kU_A/L (LOQ2 for patients 8-19) were calculated and used as cut-off for inclusion in the correlation analysis.

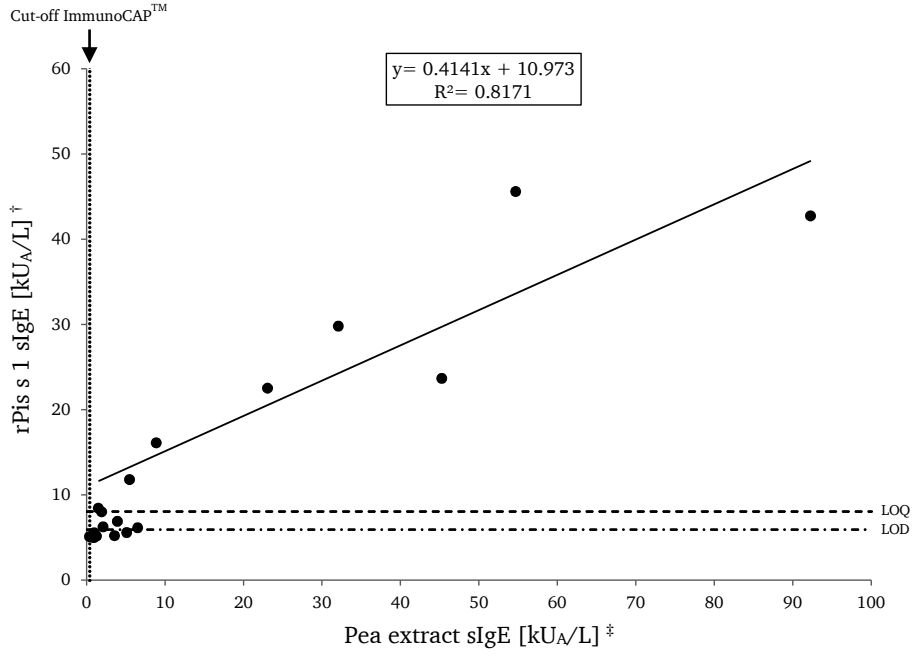


Figure 23: Correlation analysis between pea extract sIgE levels and rPis s 1 sIgE levels.

Correlation analysis was performed between pea extract sIgE levels and rPis s 1 sIgE levels > limit of quantification (LOQ). †determined by immunoblot analysis; ‡determined by ImmunoCAP™; dotted line, cut-off of ImmunoCAP™ (0.35 kU_A/L); dashed and single dotted line, mean of LOD1 and LOD2 (mean LOD= 5.95 kU_A/L). Dashed line, mean of LOQ1 and LOQ2 (mean LOQ= 8.07 kU_A/L). For correlation analysis, the respective membrane specific LOQ values were used. R^2, coefficient of determination.

In the following, the relevance of Pis s 1 and its contribution to the IgE-binding capacity of pea total protein will be discussed in more detail using the IgE immunoblot inhibition and the calculated inhibition capacity (Figure 22 and Table 26).

In pea-allergic patients 3, 10, 11 and 14, rPis s 1 was able to inhibit IgE binding to pea total protein extract almost completely. A partial inhibition of serum IgE binding could be observed in pea-allergic patients 1, 2, 4, 8, 9 and in pea-tolerant patient 16. In contrast, no inhibition could be observed in pea-allergic patient 12 and in pea-tolerant patient 17. Serum IgE of pea-allergic patients 5, 6, 7 and of pea-tolerant patients 15, 18, 19 did not show a detectable binding to pea extract in immunoblot analysis which correlated with low levels of pea extract sIgE (Figure 22 and Table 26). Consequently, no percent inhibition could be calculated for these patients, as well as for patient 13, for whom due to high background signal intensity after the addition of rPis s 1 no calculation was possible. In the remaining twelve patients (patients 1, 2, 3, 4, 8, 9, 10, 11, 12, 14, 16, 17), rPis s 1 inhibited on average 53.72% of the serum IgE binding to pea extract. For pea-allergic children the calculated average inhibition was 57.90%.

rPis s 1 was also able to inhibit IgE binding to pea extract in the lower molecular weight range between 10 and 30 kDa in several patients (patients 1, 3, 4, 8, 10, 11 and 14). The reason for this is the post-translational proteolytic cleavage of natural Pis s 1 in pea extract leading to fragments of 12.5 to 36 kDa (Gatehouse et al. 1982; Sanchez-Monge et al. 2004) that could be fully or partially inhibited by rPis s 1.

In addition, rPis s 1 could also inhibit serum IgE binding to a pea protein located between 70 and 80 kDa (Figure 22; patients 3, 4, 10 and 11). This protein might be either a dimer of Pis s 1 or potentially Pis s 2. However, in patients 1 and 16 no inhibition could be observed in this molecular weight range leading to a detectable IgE binding even after the addition of rPis s 1.

Moreover, after the addition of rPis s 1, only two patients (patients 1 and 16) still showed an IgE binding to pea proteins located in the lower molecular weight range (< 25 kDa) where MS spectrometry analysis confirmed the presence of PA1 isoforms and PA2 (Figure 24 and Table 27). Alignment of individual PA1 isoform sequences and detected isoform-specific peptides are shown in the appendix, in Figure A23 and Table A53, respectively. In addition, MS analysis identified in contrast to published data nPA1 as a proprotein version containing the propeptide linking both PA1 chains (appendix Figure A23 and Table A53).

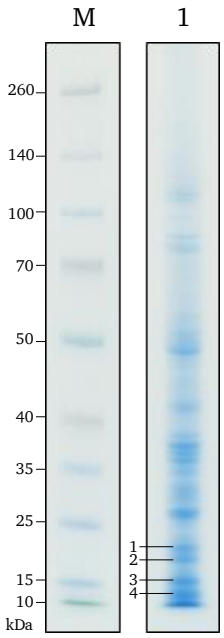


Figure 24: SDS-PAGE of pea extract for MS analysis.

Pea extract (lane 1) was analyzed under reducing conditions. Bands 1-4 were analyzed by mass spectrometry. M, Spectra™ Multicolor Broad Range Protein Ladder.

Table 27: Identification of PA1 and PA2 in pea extract.

Band	Acc. No. UP	Description	S	P	C	E
1	P08688	PA2 Albumin-2 *Pisum sativum*	4819	14	73.6	2.2
2	P08688	PA2 Albumin-2 *Pisum sativum*	10881	14	60.6	1.7
3	P08688	PA2 Albumin-2 *Pisum sativum*	21719	15	74.9	2.4
3	P62929	PA1 D Albumin-1 D *Pisum sativum*	521	2	19.2	1.2
4	P08688	PA2 Albumin-2 *Pisum sativum*	18926	12	67.1	1.4
4	P62929	PA1 D Albumin-1 D *Pisum sativum*	5376	3	36.9	2.2
4	P62930	PA1 E Albumin-1 E *Pisum sativum*	3645	2	24.6	1.0
4	P62928	PA1 C Albumin-1 C *Pisum sativum*	3231	1	6.9	4.1

Acc.No. UP, accession number of Uniprot; S, PLGS protein score; P, number of identified peptides; C, protein sequence coverage %; E, precursor RMS mass error [ppm].

Furthermore, an IgE immunoblot inhibition was performed to identify the pea protein ~14 kDa that still showed an IgE binding after the addition of rPis s 1 (appendix Figure A25). Therefore, serum from patients 1 and 16 as well as a PEI-internal serum (PEI131) from a patient sensitized to peach Pru p 3, a non-specific lipid-transfer protein (nsLTP) showing high sequence homology

to nsLTP from pea (Pis s 3), was used. By using rPA1 and natural Pru p 3 as inhibitors, it should be investigated whether the pea protein with a molecular weight of ~14 kDa was possibly PA1 or Pis s 3. However, the pea protein (~14 kDa) could not be inhibited in its IgE binding by either rPA1 or natural Pru p 3 in both pea patients (appendix Figure A25). In addition, serum PEI131 also showed IgE binding to this ~14 kDa pea protein and to an additional pea protein below (appendix Figure A25C). Using natural Pru p 3 as inhibitor, the second pea protein below 14 kDa could be inhibited indicating that pea nsLTP (Pis s 3) was presumably present in the investigated pea total protein extract.

Thus, the inhibition experiment shows that low molecular weight proteins, including PA1, PA2 and Pis s 3, that are not related to Pis s 1 fragments, play a minor role in serum IgE binding of pea-allergic children.

However, as the opposite was the case for Pis s 1, which was identified as a relevant major allergen with a high IgE-binding capacity in this study population, its biological activity was investigated in a mediator release assay using RBL-2H3 cells sensitized with a serum pool composed of pea-allergic patients 1, 3, 4, 8 and 11 (Figure 25). Both pea extract and rPis s 1 induced a comparable maximal release of approximately 64%. The amount of pea extract required for half-maximal mediator release (EC_{50}) was with 2.270 ng/well approximately one order of magnitude higher compared to rPis s 1 (EC_{50}= 0.113 ng/well).

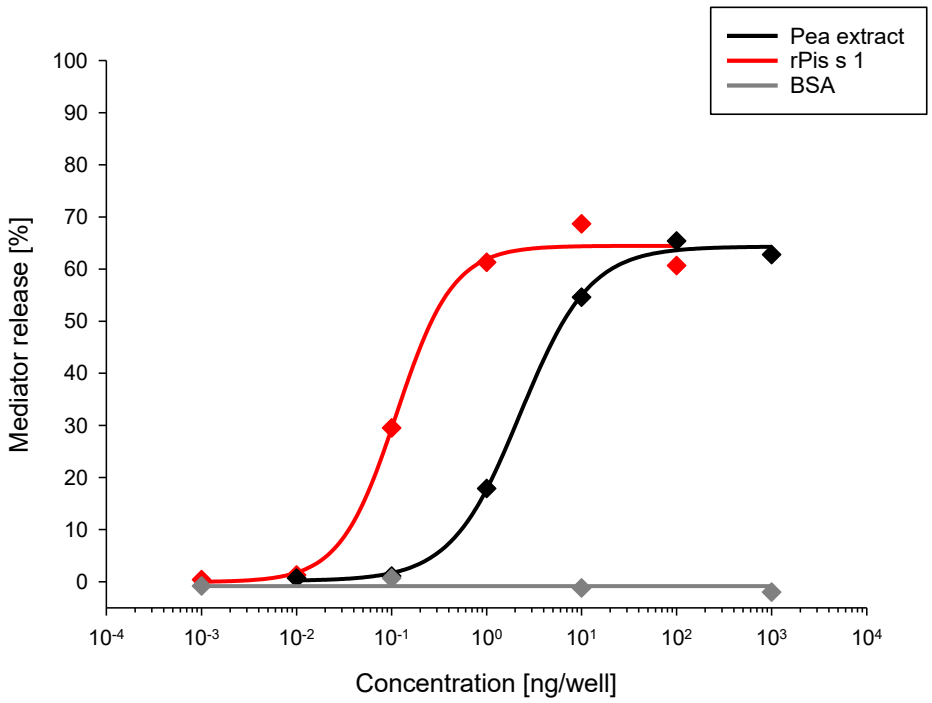


Figure 25: Mediator release induced by pea extract and rPis s 1.

Rat basophilic leukemia cells were sensitized with a serum pool of pea-allergic children (patients 1, 3, 4, 8, 11) and the mediator released induced by pea extract (black) and rPis s 1 (red) was determined. BSA (gray) was used as negative control. Depicted curves were fitted with a four parameter logistic model.

4.2.4 Differences in IgE binding at the peptide level

In the next step, all pea-allergic and tolerant children were analyzed for their differences in IgE binding to Pis s 1 at the linear peptide level.

In addition, patients 1 and 16 both showing an IgE binding to low molecular weight proteins in pea extract after inhibition with rPis s 1 (Figure 22), and in addition patient 1 also to rPA1 (Figure 21B), were analyzed for their IgE binding to peptides representing full-length PA1 and PA2. Spotting layout and peptide sequences of PA1 and PA2 are shown in the appendix, in Figure A19 and Table A48 & A49. Images of the immunodetection of the two investigated patients and the negative controls are depicted in Figure A19 in the appendix. A visual analysis of the immunodetection revealed that patient 1 showed a serum IgE binding which was above both negative controls to a limited number of peptides of PA1. This finding was in agreement with the immunoblot analysis of patient 1 and rPA1. Furthermore, this patient showed negligible or no relevant IgE binding to peptides of PA2 (Figure A19 in the appendix). For patient 16, no serum IgE binding could be detected to peptides of PA1 and PA2. This finding was also in good agreement with no IgE binding to rPA1 and rPA2 in immunoblot analysis.

As no significant serum IgE binding could be detected to PA1 and PA2 at the protein and linear peptide level, no detailed Z-score calculation was performed and both proteins were not further investigated and will not be further discussed in this results section.

By contrast, Pis s 1 bound serum IgE of the majority of pea-allergic patients and therefore, was analyzed in detail for its IgE binding at the peptide level. Similar to the peanut project, peptides representing full-length mature Pis s 1 were spotted in quadruplicate and subsequently, successful spotting was confirmed by Coomassie staining of randomly chosen array slides (Figure 26A and 26B). As an example, the IgE immunodetection using serum of patient 11, who showed a strong IgE binding to peptides of Pis s 1, is illustrated in Figure 26C.

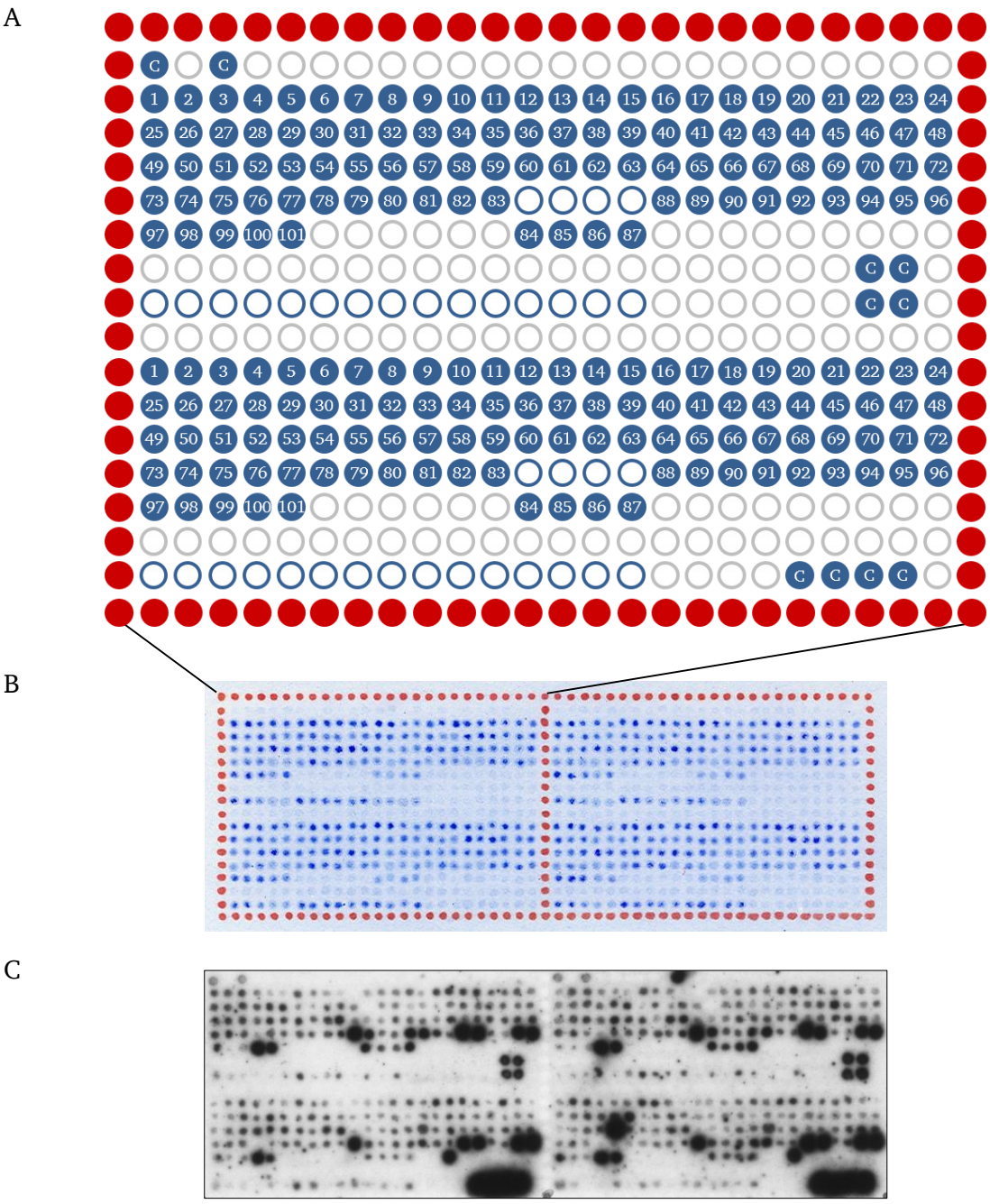


Figure 26: Spotting layout, Coomassie-staining and IgE immunodetection of Celluspot™ multipeptide microarray displaying Pis s 1.

(A) Spotting layout of Pis s 1 multipeptide microarray. For simplification, only the left segment of the multipeptide microarray is shown. Full-length sequence of Pis s 1 is represented by 101 peptides that were spotted (each 0.04 μl) in duplicate on each segment leading to quadruplicate peptide presentation on the whole array. Biotinylated control peptides, abbreviated by a "C", were spotted in duplicate in different dilutions (top left, middle right and bottom right on each array element) and were used as position markers. Gray empty spots represent blank spots composed of peptide printing buffer (DMSO). Blue empty spots represent internal control peptides not relevant for peptide analysis. (B) Successful spotting was verified by staining with Coomassie Brilliant Blue G250. (C) IgE immunodetection using serum of a pea-allergic child (patient 11) after 2 min exposure.

In total, 101 peptides, that represented the full-length mature sequence of Pis s 1, were spotted. Again, quadruplicate peptides should minimize the influence of outliers, and positive controls should help to simplify the peptide analysis using Spotfinder™ software. As shown in Figure 26C, patient 11 showed a strong serum IgE binding to the peptides of Pis s 1 and especially to peptides located at the carboxy-terminal part.

To identify differences between pea-allergic and tolerant children with regard to their serum IgE binding to Pis s 1 peptides, all patients were individually analyzed for their IgE-binding propensity. After the immunodetection, the signal intensity of each Pis s 1 peptide, expressed as Z-score, was calculated. Similar to the peanut project, serum IgE binding to a peptide was defined as positive if its Z-score was > 2 after subtraction of the maximum Z-score of the controls. As controls in microarray analysis, sera from five atopic (serum A-E) and five non-atopic (serum F-J) controls were used.

The images of the IgE immunodetection as well as calculated Z-scores of controls and of pea study subjects after control subtraction are listed in the appendix (Figure A17 and Table A42-A46).

After Z-score calculation, the number of positive IgE-bound peptides was calculated for every patient (Table 28). Serum IgE of all (14/14) pea-allergic children and of 4/5 (80%) pea-tolerant children bound to at least one peptide of Pis s 1. However, serum IgE of pea-allergic children bound to more peptides (median of IgE-bound peptides: 31) compared to serum IgE of pea-tolerant children (median of IgE-bound peptides: 1), this reflected higher IgE-binding diversity of pea-allergic children.

A comparison of the IgE binding to rPis s 1 in immunoblot analysis with the IgE binding to linear peptides of Pis s 1 in microarray analysis revealed that patients with a rPis s 1 sIgE level below the LOD, meaning with no detectable serum IgE binding to rPis s 1 (patients 7, 9, 12, 15, 17-19), tended to recognize fewer peptides as patients showing a positive serum IgE binding to rPis s 1. Patient 7 was one exception, as no IgE binding to rPis s 1 and to pea total protein was detectable in immunoblot analysis, whereas in microarray analysis IgE binding to peptides of Pis s 1 could be detected. Allergic patients who recognized the highest number (> median) of Pis s 1 peptides (patients 1, 4, 6, 7, 8, 10, 11) tended to have higher rPis s 1 sIgE levels. Exceptions here were the above-mentioned patient 7 and patient 6 who recognized a high number of peptides but had a very low rPis s 1 sIgE level and showed no IgE binding to pea total protein in immunoblot analysis.

Table 28: Number of positive serum IgE-bound peptides of Pis s 1.

Positive peptide IgE binding was defined if the Z-score exceeds 2 after control subtraction.

Patient No.	Number of IgE-bound peptides of Pis s 1	Patient No.	Number of IgE-bound peptides of Pis s 1
1	49	11	70
2	4	12	2
3	24	13	16
4	88	14	2
5	16	15	0
6	73	16	25
7	43	17	1
8	85	18	1
9	5	19	2
10	37		

Afterwards, the frequency of recognition by pea-allergic and pea-tolerant patients was calculated for every peptide of Pis s 1 (Figure 27). As shown in Figure 27, pea-allergic patients showed higher IgE-diversity compared to pea-tolerant patients. Moreover, pea-allergic patients showed a maximum IgE-binding frequency of 93% (13/14 patients) to peptides 95 and 96 of Pis s 1. However, these two peptides were also recognized by 20% (1/5) of pea-tolerant patients. Nevertheless, there were different peptide stretches and individual peptides that were only recognized by pea-allergic children.

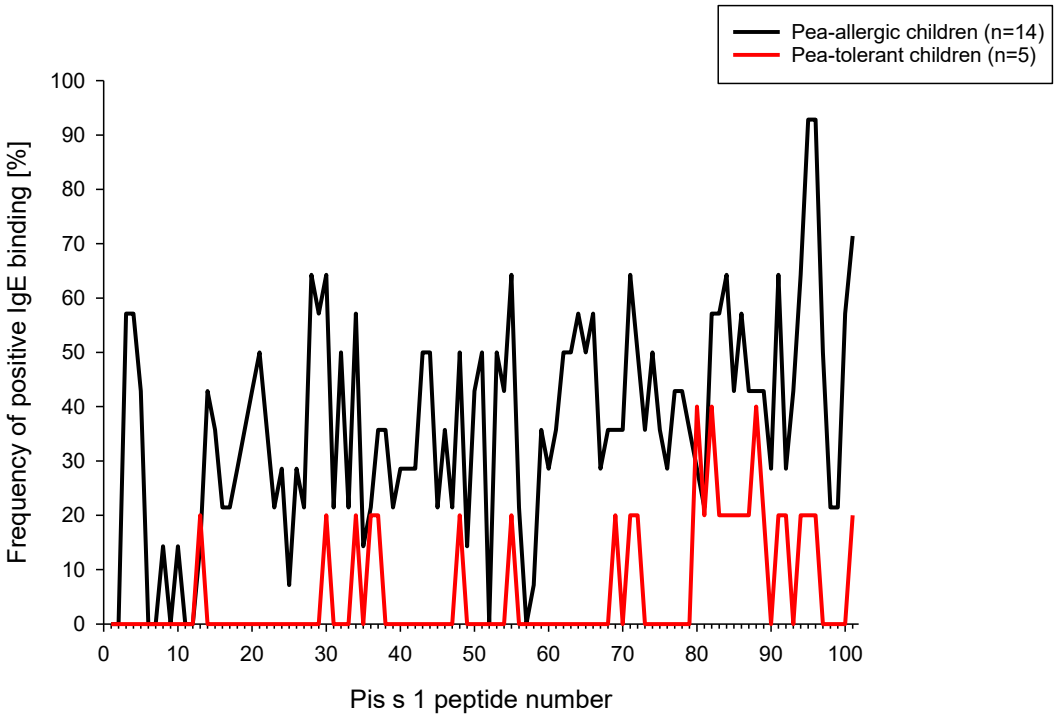


Figure 27: IgE-binding frequencies of pea-allergic and tolerant patients to peptides of Pis s 1.

Comparison of the IgE-binding frequencies of pea-allergic (black) and tolerant (red) children to peptides of Pis s 1. Serum IgE binding to a peptide was considered positive if Z-score was > 2.

4.2.5 Identification of candidate diagnostic peptides of Pis s 1

For the identification of candidate diagnostic peptides of Pis s 1 specific for pea allergy the same established selection criteria as in the peanut project were applied. Briefly, specific candidate peptides containing distinct amino acid sequences should be exclusively recognized by pea-allergic children with high signal intensities and high frequencies. For verifying the specificity of peptide IgE binding, inhibition experiments using two serum pools and 30 μg rPis s 1 were performed. The two serum pools were composed of patients 1, 4, 8 (pool 1) and patients 3, 10, 11, 13 (pool 2). Sera within one pool showed comparable IgE-binding pattern to the peptides of Pis s 1. Images of the IgE inhibition experiments and calculated Z-scores can be found in the appendix (Figure A18 and Table A47). Peptides fulfilling the mentioned criteria were further narrowed and finally peptides with an IgE-binding frequency of $\geq$ 42.85% were selected as candidate diagnostic peptides. This percentage value resulted from the calculation of the median IgE-binding frequency of the preselected peptides.

In total, eleven peptides distributed over the entire Pis s 1 primary structure could be identified fulfilling the above defined selection criteria for candidate diagnostic peptides that should be specific for pea allergy (Figure 28, blue bars).

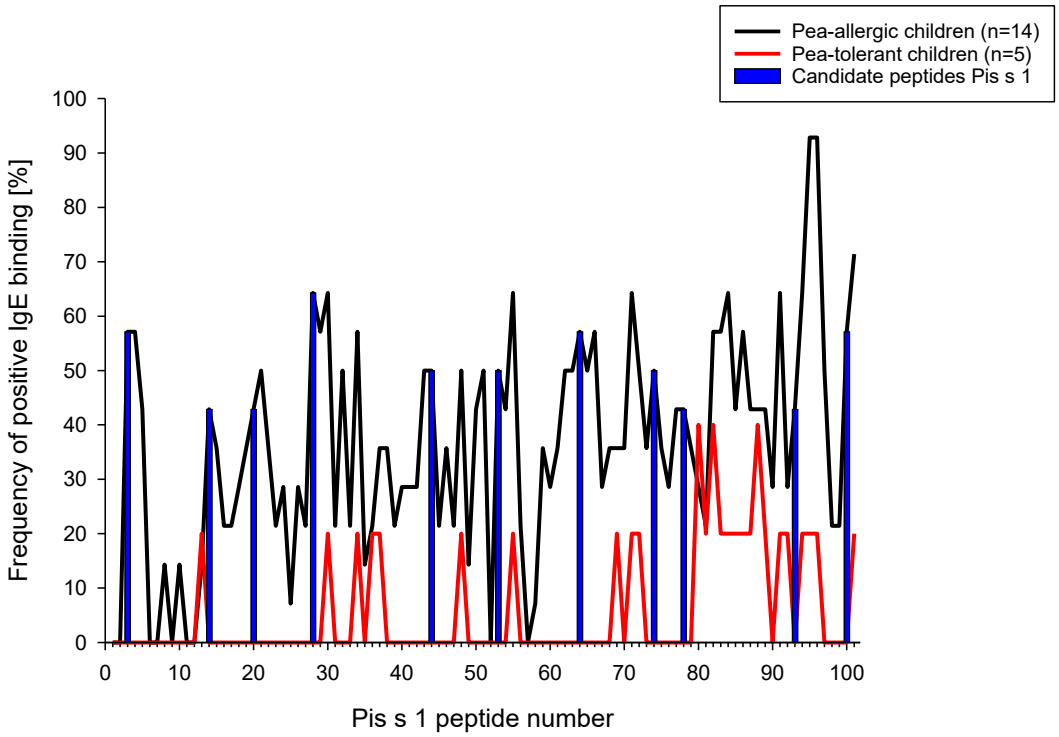


Figure 28: Identified peptides of Pis s 1 fulfilling the selection criteria for candidate diagnostic peptides.
Comparison of the IgE-binding frequencies of pea-allergic (black) and tolerant (red) children to peptides of Pis s 1. Serum IgE binding to a peptide was considered positive if Z-score was > 2. Identified candidate diagnostic peptides within this study population specific for pea allergy are shown in blue bars.

The identified candidate diagnostic peptides were peptide 3, 14, 20, 28, 44, 53, 64, 74, 78, 93 and 100. These candidate diagnostic peptides were recognized by 57%, 43%, 43%, 64%, 50%, 50%, 57%, 50%, 43%, 43% and 57% of pea-allergic children, respectively. Pea-tolerant patients did not show, according to the selection criteria, an IgE binding to the eleven candidate diagnostic peptides. Individual serum IgE binding of pea-allergic and tolerant children to the selected candidate diagnostic peptides is shown in Table 29. Serum IgE of 13/14 (93%) pea-allergic children bound to at least one peptide of the eleven candidate diagnostic peptides. Only serum IgE of patient 14 did not bind to any of the identified candidate diagnostic peptides. The number of recognized candidate diagnostic peptides varied in pea-allergic children between 0 and 11 peptides with a median number of 6 recognized peptides.

Table 29: IgE binding of pea-allergic and tolerant children to identified candidate diagnostic peptides of Pis s 1.

Eleven peptides could be identified as candidate diagnostic peptides of Pis s 1. X depicts positive serum IgE binding to candidate peptide; - depicts negative serum IgE binding.

Patient No.	Pis s 1 P3	Pis s 1 P14	Pis s 1 P20	Pis s 1 P28	Pis s 1 P44	Pis s 1 P53	Pis s 1 P64	Pis s 1 P74	Pis s 1 P78	Pis s 1 P93	Pis s 1 P100
1	X	X	-	X	-	X	X	X	X	-	X
2	-	-	-	-	-	-	-	X	-	-	-
3	-	-	-	X	X	-	X	-	X	-	X
4	X	X	X	X	X	X	X	X	X	X	X
5	-	-	-	X	-	X	-	-	-	-	-
6	X	X	X	X	X	X	X	X	X	X	-
7	X	X	X	X	X	X	X	-	-	X	X
8	X	X	X	X	X	X	X	X	X	X	X
9	-	-	-	-	-	-	-	-	-	-	X
10	X	-	-	X	-	-	X	X	-	X	X
11	X	X	X	X	X	X	X	X	X	X	X
12	-	-	X	-	-	-	-	-	-	-	-
13	X	-	-	-	X	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-

The amino acid sequences of the eleven identified candidate diagnostic peptides are listed in Table 30. Peptides like peptide 28, 44 and 78 contained a high portion (7 to 8 amino acid residues) of charged amino acid residues and peptide 53 was especially enriched in polar amino acid residues compared to other candidate diagnostic peptides.

Table 30: Identified candidate diagnostic peptides of Pis s 1.

Amino acid sequence of identified candidate diagnostic peptides of Pis s 1.

Candidate peptides of Pis s 1	Amino acid sequence
Peptide 3	F-I-F-K-S-N-R-F-Q-T-L-Y-E-N-E
Peptide 14	S-K-P-H-T-L-F-L-P-Q-Y-T-D-A-D
Peptide 20	A-T-L-T-V-L-K-S-N-D-R-N-S-F-N
Peptide 28	A-N-R-D-D-N-E-D-L-R-V-L-D-L-A
Peptide 44	Q-Q-E-Q-E-P-Q-H-R-R-S-L-K-D-R
Peptide 53	S-K-N-A-K-S-S-S-K-K-S-V-S-S-E
Peptide 64	Q-L-Q-D-L-D-I-F-V-N-S-V-D-I-K
Peptide 74	F-E-L-V-G-Q-R-N-E-N-Q-G-K-E-N

Peptide 78	K-E-E-E-Q-E-E-E-T-S-K-Q-V-Q-L
Peptide 93	G-E-E-D-N-V-I-S-Q-V-E-R-P-V-K
Peptide 100	L-L-K-N-Q-K-Q-S-Y-F-A-N-A-Q-P

Figure 29 depicts the molecular surface presentation of the eleven identified candidate diagnostic peptides on Pis s 1. The eleven peptides were distributed over the entire molecular surface of Pis s 1. No predominant location of the eleven candidate diagnostic peptides could be observed within this pea study group.

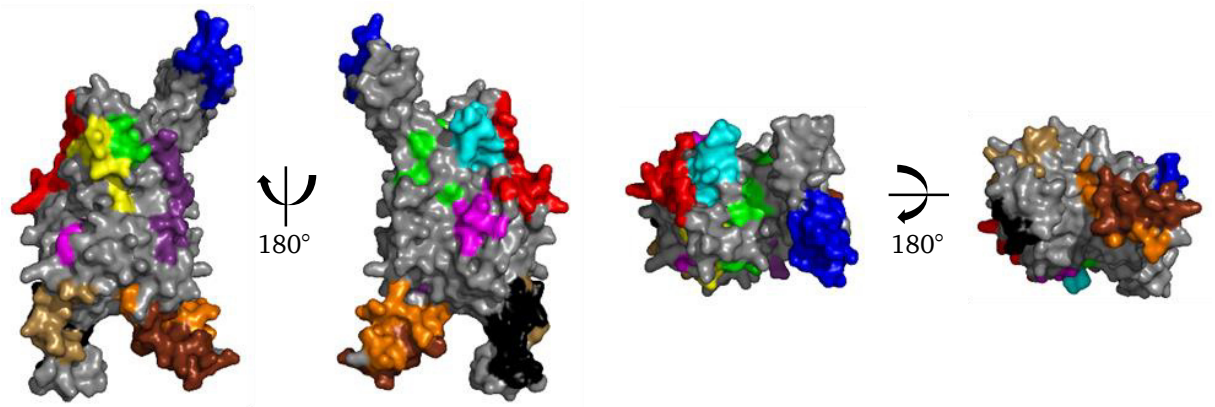


Figure 29: Surface presentation of identified candidate diagnostic peptides of Pis s 1.

Molecular surface presentation of the eleven candidate diagnostic peptides of Pis s 1. P3, violetpurple; P14, green; P20, cyan; P28, yellow; P44, blue; P53, red; P64, magenta; P74, orange; P78, brown; P93, black; P100, sand. Images were generated using pdb 1UIJ and PyMOL.

4.2.6 Diagnostic value of candidate diagnostic peptides in comparison to full-length rPis s 1 and pea extract

Finally, an ROC curve analysis was performed in order to determine and compare the diagnostic value of pea total protein extract, full-length rPis s 1 and identified candidate diagnostic peptides. Prior to ROC curve analysis rPis s 1 sIgE levels (kU_A/L) were subtracted by two times the signal intensity of the non-allergic control serum N1 or N2 resolved for sIgE (as described in chapter 3.2.8.7). In addition, of the eleven candidate diagnostic peptides, the maximum Z-score was calculated. The calculated maximum Z-score of the eleven peptides for each individual study subject is listed in Table 31.

Table 31: Maximum Z-scores of candidate diagnostic peptides of Pis s 1.

For each patient, the maximum Z-score was calculated of the eleven candidate peptides of Pis s 1. For this, Z-scores after subtraction of the controls were used.

Patient No.	Max. Z-score candidate peptides Pis s 1 (n=11 peptides)	Patient No.	Max. Z-score candidate peptides Pis s 1 (n=11 peptides)
1	8.456176757	11	28.93225251
2	4.390234033	12	2.513176118
3	13.75090006	13	2.794986271
4	33.82082034	14	1.672636293
5	5.107092898	15	-1.208033918
6	13.19017001	16	1.992643739
7	6.94685405	17	0.593583355
8	32.05170301	18	-0.850040617
9	2.05895219	19	-0.115452683
10	5.604967253		

The ROC curves and the AUC values of pea extract, rPis s 1 and candidate diagnostic peptides are shown in Figure 30. sIgE to pea extract and rPis s 1 had an AUC of 0.81 and 0.86, respectively. Candidate diagnostic peptides had an AUC of 0.99 and represented a perfect discriminative ability between pea-allergic and tolerant children.

sIgE to pea extract as well as sIgE to rPis s 1 reached at a specificity of 100% a sensitivity of 64%. Using positive IgE binding to rPis s 1 as cut-off, a specificity of 80% and a sensitivity of 79% could be achieved. In contrast, the eleven identified candidate diagnostic peptides reached at a specificity of 100% the highest sensitivity of 93%. Consequently, candidate diagnostic peptides increased the sensitivity (79% vs. 93%) and the specificity (80% vs. 100%). The identified candidate diagnostic peptides enabled, in comparison to the IgE binding to full-length rPis s 1, the serum IgE binding of three additional patients (patients 7, 9 and 12). Serum IgE of pea-allergic patient 14, on the other hand, bound to full-length rPis s 1, but not to any of the eleven candidate diagnostic peptides.

In a last analysis step, it should be analyzed whether the eleven peptides could be further narrowed while keeping the specificity (100%) and the sensitivity (93%). An analysis of the minimum number of peptides required to maintain 93% sensitivity (13/14 pea-allergic patients) revealed a minimum number of five peptides. Peptide 20 was required for the detection of patient 12, peptide 74 for patient 2 and peptide 100 for patient 9. Using these three peptides resulted in a sensitivity of just 79%, as patients 5 and 13 did not show an IgE binding to any of these three peptides. Patient 5 showed only an IgE binding to peptides 28 and 53 and patient 13 to peptides 3 and 44. Of these two peptide pairs, the two peptides with the highest frequency of recognition in pea-allergic children were finally selected. This resulted in the

selection of peptides 3 and 28. An ROC curve analysis using the maximum Z-score of these five peptides (peptides 3, 20, 28, 74 and 100) resulted in an AUC of 0.99 (95% CI: 0.95-1.03) which was identical to the AUC of the eleven candidate diagnostic peptides (data not shown).

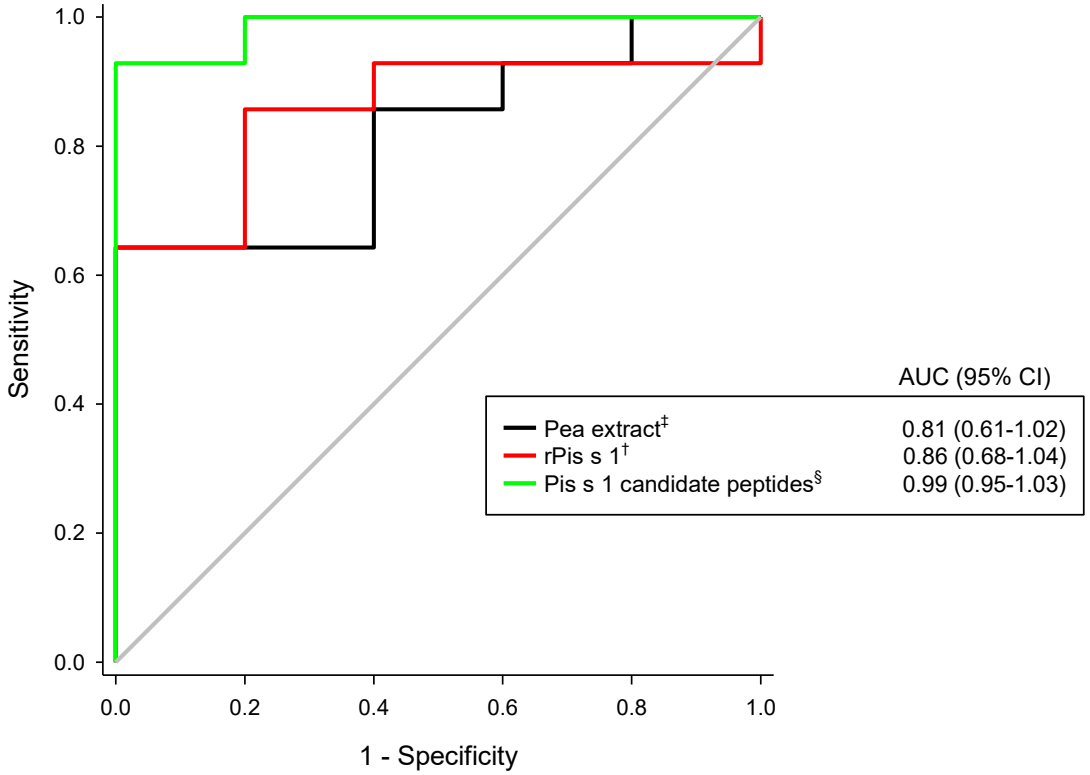


Figure 30: Receiver operating characteristic (ROC) curve analysis of sIgE to pea total protein extract, full-length recombinant Pis s1 and identified candidate diagnostic peptides.

ROC curves of sIgE to pea extract, rPis s 1 and candidate peptides of Pis s 1 are shown in black, red and green, respectively. sIgE to pea extract was determined by ImmunoCAP™ analysis (‡) and sIgE to rPis s 1 was quantified by immunoblot analysis (†). For ROC curve analysis of candidate diagnostic peptides, calculated maximum Z-score was used. Z-scores were determined by microarray analysis (§). The gray diagonal line (AUC 0.5) represents a test without discriminatory ability. AUC, area under the ROC curve; CI, confidence interval.

4.3 Soybean project

4.3.1 Study population

Included in the soybean project were sera from twenty-one children (median age 3 years), in total. Characteristics of soybean patients are listed in Table 32. The inclusion criterion was, similar to the both other projects, a soybean sIgE level of ≥ 0.35 kU_A/L. In six of these twenty-one children (patients 1-6) soybean allergy was confirmed by oral food challenge. 2/6 underwent a double-blind placebo-controlled food challenge (DBPCFC) and 4/6 an open food challenge (OFC). In the remaining fifteen children (patients 7-21) soybean allergy was excluded and thus, soybean tolerance, despite sensitization, was confirmed by oral food challenge (11/15 DBPCFC and 4/15 OFC). All six allergic patients showed during food challenge moderate (grade II/III) soybean-related symptoms. Soybean sIgE level ranged in allergic children from 1.51 to 93.20 kU_A/L. The calculated median soybean sIgE level was 15.85 kU_A/L in allergic children which was elevated compared to tolerant children having a median soybean sIgE level of 2.60 kU_A/L (range 0.60 to 42.70 kU_A/L).

Table 32: Clinical characteristics of the soybean study population.

Patients allergic to soybean (patients 1-6) and patients sensitized to soybean without clinical symptoms (patients 7-21).

Patient No.	Age (y)*/sex	Clinical evidence	Symptoms to soybean	Severity grading#	Total IgE (kU_A/L)‡	Soybean sIgE (kU_A/L)‡
1	2/m	OFC	GU	II	219.00	4.16
2	11/m	OFC	N, rGC	II	n.d.	93.20
3	6/f	DBPCFC	N, IT	III	n.d.	1.51
4	5/m	OFC	GU, I	II	417.00	11.30
5	2/m	OFC	GU, I	II	695.00	20.40
6	2/m	DBPCFC	CU, N, RC	III	238.00	24.90
7	1/m	DBPCFC	none	-	404.00	2.60
8	1/m	DBPCFC	none	-	259.00	0.72
9	6/f	DBPCFC	none	-	953.00	42.70
10	4/m	DBPCFC	none	-	202.00	1.64
11	2/f	OFC	none	-	275.00	0.60
12	4/m	DBPCFC	none	-	82.00	1.52
13	6/m	DBPCFC	none	-	n.d.	3.27
14	2/m	DBPCFC	none	-	n.d.	2.61
15	3/m	DBPCFC	none	-	n.d.	0.81
16	2/m	DBPCFC	none	-	n.d.	0.61
17	4/f	DBPCFC	none	-	n.d.	1.38
18	10/f	DBPCFC	none	-	n.d.	7.35
19	2/m	OFC	none	-	1094.00	3.00

20	1/f	OFC	none	-	1962.00	6.10
21	7/m	OFC	none	-	n.d.	2.86

*Age at time of blood sampling; f, female; m, male; n.d., not determined; DBPCFC, double-blind placebo-controlled food challenge; OFC, open food challenge; CU, contact urticaria; GU, generalized urticaria; I, itching; IT, itching throat; N, nausea; RC, rhinoconjunctivitis; rGC, reduced general conditions; #severity grading according to the grading system developed by Sampson (Sampson 2003); ‡ determined by ImmunoCAP™.

4.3.2 Generation and physicochemical characterization of rGly m 5.03 and rGly m 8

Both proteins, the β subunit of Gly m 5 (Gly m 5.03) and Gly m 8, could be successfully expressed using *Pichia pastoris* and were purified from cell culture supernatant using IMAC and SEC. rGly m 8 was expressed as proprotein composed of two chains linked by a 17 amino acid residue propeptide (for sequence information see Figure A11 in the appendix).

The identity of both recombinant proteins was confirmed by mass spectrometry analysis (Table 33). Sequence coverages for rGly m 5.03 and rGly m 8 of 45.5% and 30.6% were determined, respectively.

Table 33: Identity confirmation of recombinant soybean proteins by mass spectrometry analysis.

Protein	Accession number#	PLGS protein score	Number of identified peptides	Sequence coverage	Mass error*
rGly m 5.03	PEI078	4026	20	45.5%	19.9
rGly m 8	PEI075	821	6	30.6%	6.2

#PEI, internal accession number; *precursor RMS mass error [ppm].

According to Coomassie-stained SDS-PAGE shown in Figure 31A, rGly m 5.03 (49.3 kDa) in lane 1 and rGly m 8 (17.5 kDa) in lane 2 showed high purity suitable for further IgE binding analyses. Weak bands < 49 kDa in lane 1 were degradation products of rGly m 5.03 and additional weak bands between 140 and 260 kDa were possibly trimeric or polymeric rGly m 5.03.

CD spectroscopy indicated structural integrity of both recombinant soybean proteins (Figure 31B and 31C) and revealed for rGly m 5.03 (Figure 31B) a CD spectrum comparable to pea 7S globulin rPis s 1 (Figure 20B). The CD spectrum showed a minimum at ~217 nm and a maximum at ~197 nm. In addition, the CD spectrum of rGly m 8 (Figure 31C) was comparable to peanut 2S albumins Ara h 2 (Figure 2B) and pea albumin 1 (Figure 20B). Here, the typical α-helical characteristics, two minima at ~208 nm and ~222 nm and a maximum at ~193 nm, could be detected. Using dynamic light scattering, the hydrodynamic radii (R_H) of both soybean allergens was measured. For rGly m 5.03 a R_H of 7.4 ± 1.2 nm was detected suggesting a

trimeric protein in solution comparable to rPis s 1. Whereas, rGly m 8 (R_H of 2.6 ± 0.4 nm), comparable to the other investigated 2S albumins, seemed to be present as a monomeric protein in solution.

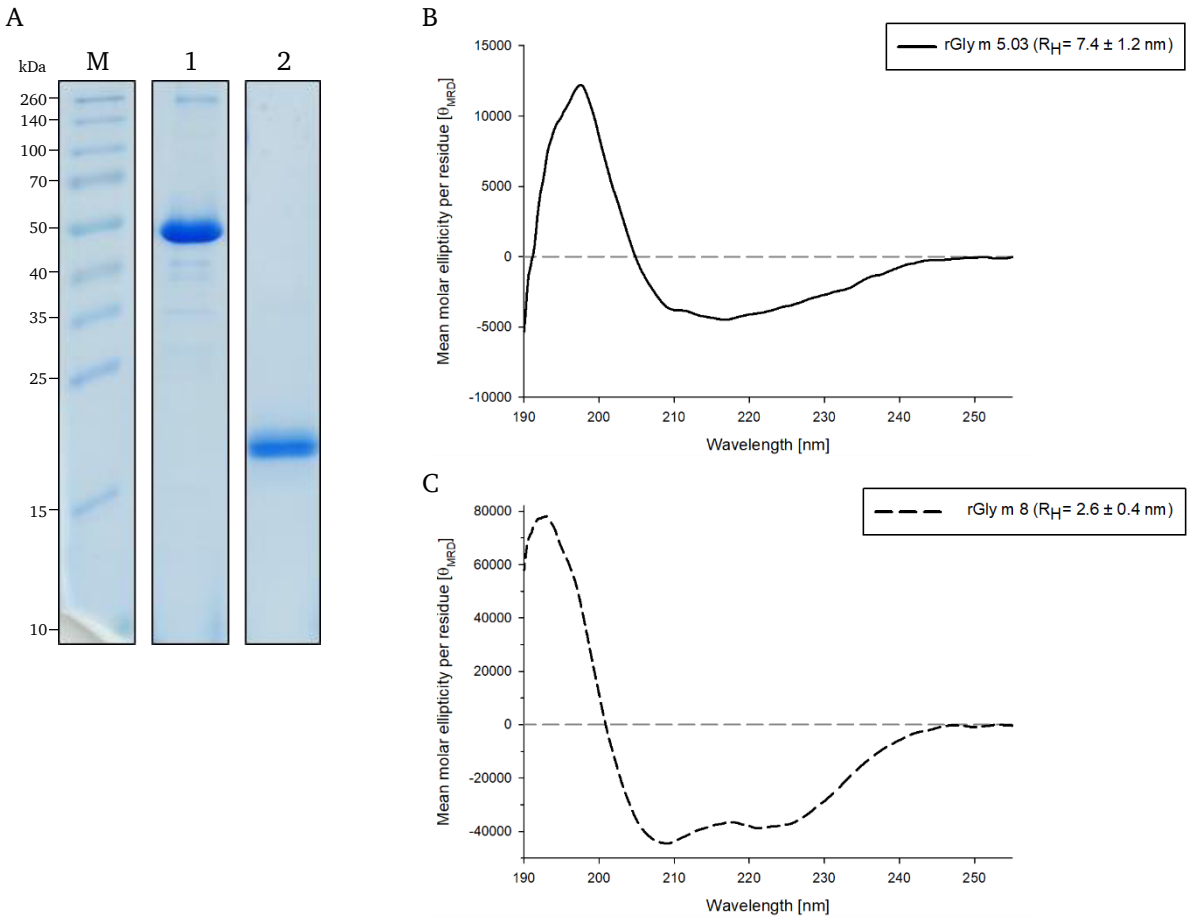


Figure 31: Purity and physicochemical characterization of rGly m 5.03 and rGly m 8.

(A) Coomassie-stained SDS-PAGE of rGly m 5.03 (lane 1; 7 μg/cm) and rGly m 8 (lane 2; 1.5 μg/cm). Protein samples were analyzed under reducing conditions. M, Spectra^{TM} Multicolor Broad Range Protein Ladder. (B) Far-UV CD-spectrum (190-255 nm) of rGly m 5.03 (solid line) at 3 μM. (C) Far-UV CD-spectrum (190-255 nm) of rGly m 8 (dashed line) at 2 μM. The inset depicts the hydrodynamic radius (R_H) ± SD.

4.3.3 Relevance of Gly m 5.03 and Gly m 8

In order to determine the relevance of both proteins in this study population, they were analyzed for their IgE-binding properties using immunoblot analysis (Figure 32). Patients' sIgE levels were densitometrically quantified and, comparable to the two other legume projects, serum IgE binding was considered positive when the sIgE level exceeds the LOD (2x sIgE level of non-allergic control serum N). As neither an allergic nor a tolerant patient showed obviously a serum IgE binding to rGly m 8 (Figure 32B), densitometric quantification of rGly m 8 sIgE levels was omitted.

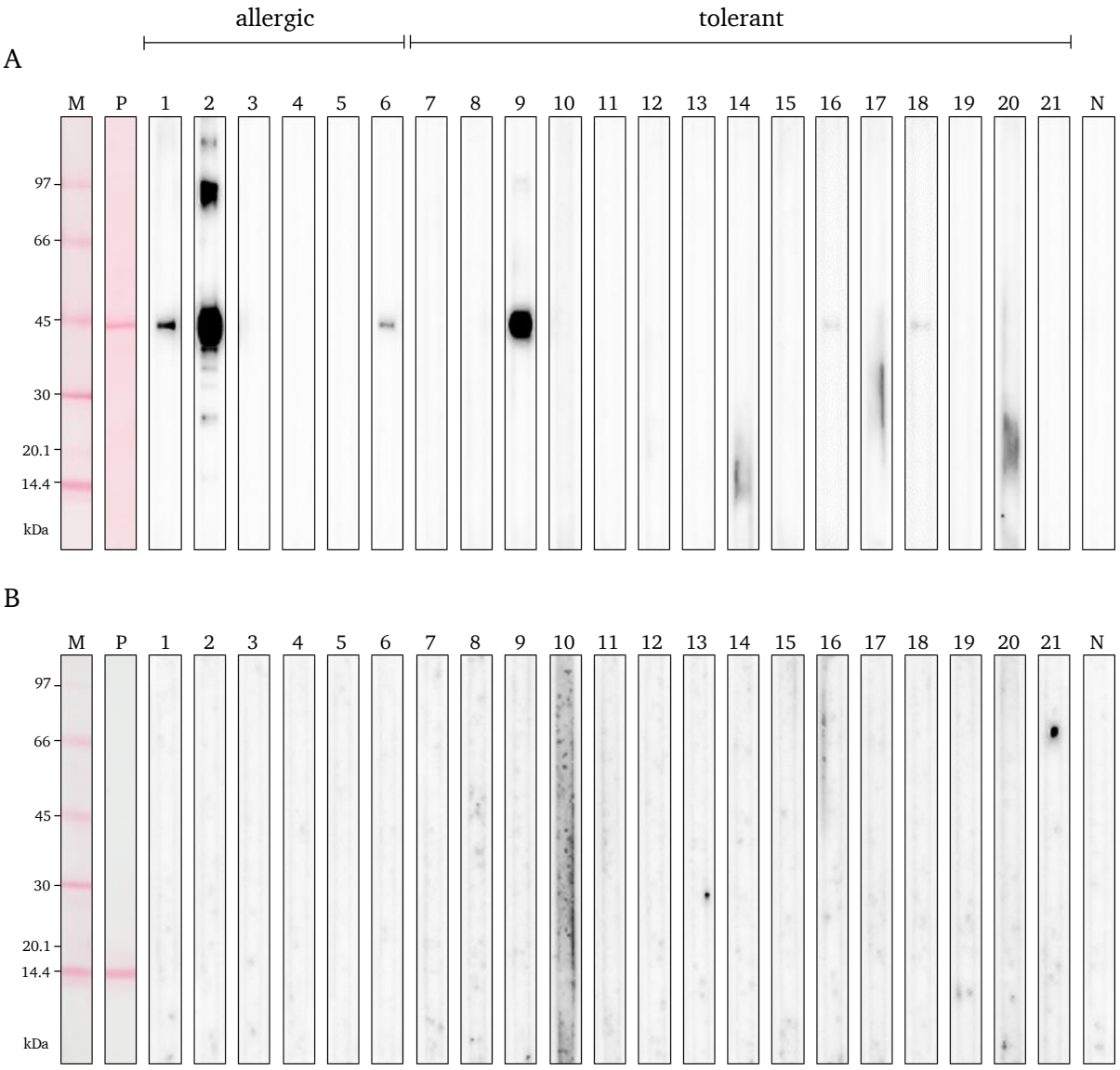


Figure 32: Serum IgE binding of soybean-allergic and sensitized but tolerant children to rGly m 5.03 and rGly m 8.

IgE binding of sera from soybean-allergic children (lane 1-6, patients' sera 1-6 according to Table 32) and tolerant children (lane 7-21, patients' sera 7-21 according to Table 32) to rGly m 5.03 (A) and to rGly m 8 (B) after 1 min and 4 min exposure, respectively. Protein samples were analyzed under reducing conditions. M, low-molecular weight marker; P, total protein stained with Ponceau S; N, non-allergic control serum.

rGly m 5.03, in contrast, showed serum IgE binding (Figure 32A) and patients' quantified rGly m 5.03 sIgE levels are listed in Table 34. All patients were analyzed for their serum IgE binding on one immunoblot membrane and the calculated LOD was 1.104 kU_A/L. Considering the LOD, serum IgE of 3/6 (50%; patients 1, 2, 6) soybean-allergic children and of 3/15 (20%; patients 9, 16, 18) soybean-tolerant children bound to rGly m 5.03. Patient 2 also recognized degradation products of rGly m 5.03 and di/trimeric rGly m 5.03.

Quantified rGly m 5.03 sIgE levels (> LOD) ranged from 4.15 to 58.02 kU_A/L (median 10.05 kU_A/L) in the allergic group and from 1.72 to 37.20 kU_A/L (median 1.99 kU_A/L) in the tolerant group.

Table 34: Quantified rGly m 5.03 sIgE levels of soybean-allergic and sensitized but tolerant children.
Densitometrically quantified rGly m 5.03 sIgE levels of soybean-allergic (patients 1-6) and sensitized but tolerant children (patients 7-21). N, non-allergic control serum.

Patient No.	rGly m 5.03 sIgE (kU_A/L)†	Patient No.	rGly m 5.03 sIgE (kU_A/L)†
1	10.050	12	(0.919)
2	58.016	13	(0.594)
3	(0.749)	14	(1.073)
4	(0.891)	15	(0.891)
5	(0.790)	16	1.717
6	4.149	17	(1.011)
7	(0.800)	18	1.991
8	(0.816)	19	(0.973)
9	37.202	20	(1.050)
10	(1.033)	21	(0.785)
11	(0.789)	N	0.552

† determined by immunoblot analysis; () sIgE levels in parenthesis are below method cut-off (LOD).

In addition, serum IgE binding to total soybean protein was analyzed and the result of the IgE immunoblot is shown in Figure 33. Soybean-allergic patients 2 and 6 as well as soybean-tolerant patients 8, 9 and 20 showed a strong IgE binding to soybean total protein. In contrast, no or only minor IgE binding could be detected for patients 3-5, 7, 11-16, 19 and 21 which, for the majority of the mentioned patients, correlated with low levels of soybean extract sIgE (Table 32). However, soybean-allergic patients 4 and 5 had moderate levels of sIgE (11.3 and 20.4 kU_A/L, respectively) to soybean extract, nevertheless only minor serum IgE binding to total soybean extract could be identified. This could be caused by the different soybean extracts used in this study and in the ImmunoCAP™ and potentially underrepresented or missing allergens. The native β subunit of Gly m 5 (Gly m 5.03) has a molecular weight of approximately 48 kDa. Soybean-allergic patients 1, 2, 6 and soybean-tolerant patients 8, 9 and, albeit weak, patients 12 and 18, showed IgE binding to a soybean protein at > 45 kDa, which was presumably Gly m 5.03. Except for patients 8 and 12, this IgE binding was in accordance with a positive IgE binding to rGly m 5.03 determined in immunoblot analysis (Figure 32A). In addition, patient 16 showed a weak IgE binding to rGly m 5.03 which could not be detected using soybean extract with nGly m 5.03.

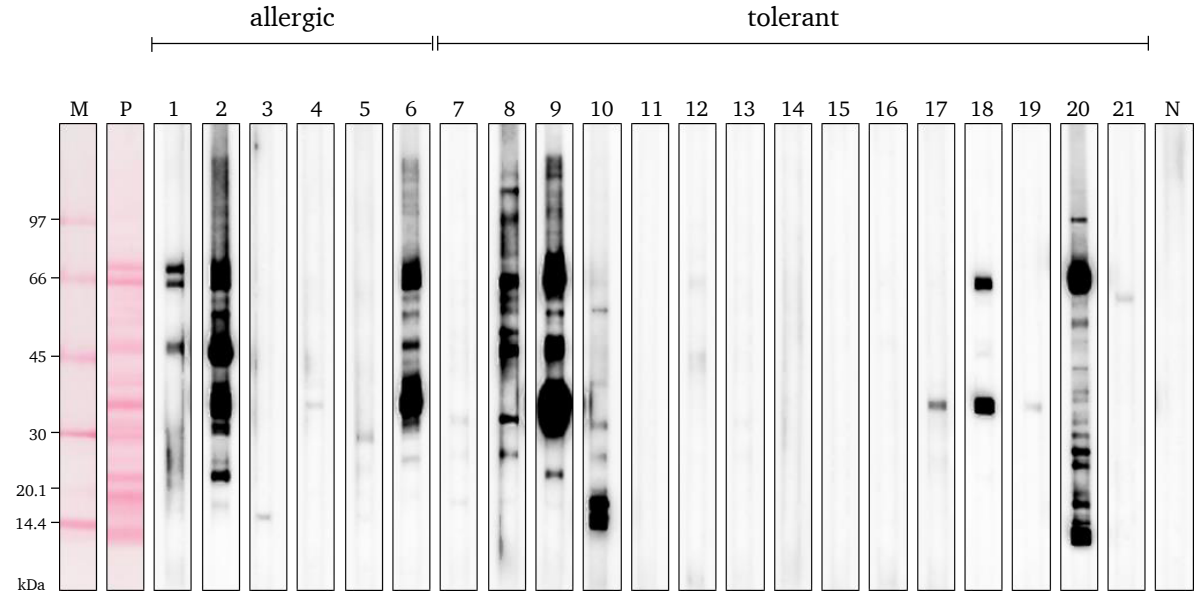


Figure 33: Serum IgE binding of soybean-allergic and sensitized but tolerant children to soybean total protein extract.

IgE binding of sera from soybean-allergic children (lane 1-6, patients' sera 1-6 according to Table 32) and tolerant children (lane 7-21, patients' sera 7-21 according to Table 32) to soybean extract. Soybean extract was analyzed under reducing conditions. The detection is shown after 1 min of exposure (except for patient 2: 15 sec of exposure). M, low-molecular weight marker; P, total protein stained with Ponceau S; N, non-allergic control serum.

In general, no dominant serum IgE binding to soybean proteins located in the lower molecular weight range could be detected in either soybean-allergic or tolerant patients. Only soybean-tolerant patients 10 and 20 showed a significant serum IgE binding to soybean proteins below 25 kDa, where MS analysis confirmed the presence of nGly m 8 in multiple bands (Figure 34 lane 1 and Table 35 bands 1-4).

rGly m 8 was investigated under reducing conditions and as one peptide chain that includes a propeptide. Both characteristics may impact the IgE-binding properties of rGly m 8 compared to natural Gly m 8 in soybean extract which is composed of two chains linked by disulfide bonds. Accordingly, a native PAGE of the soybean extract was performed in order to present natural Gly m 8 in its native conformation.

MS analysis of soybean extract run under native conditions confirmed the identity of nGly m 8 in band 5 (Figure 34 lane 2 and Table 35 band 5). Moreover, MS analysis identified in band 5 as well as in band 1-4 N- and C-terminal peptides suggesting the presence of full-length mature nGly m 8 without propeptide. Identified peptides of nGly m 8 in bands 1-5 are listed in the appendix, in Table A54.

However, also using native PAGE and IgE immunoblot analysis, no serum IgE binding could be detected in soybean-allergic patients in the area where MS analysis identified nGly m 8. Even

after a long exposure (10 min), only soybean-tolerant patients 10 and 20 showed a weak IgE binding to a soybean protein located in the area of nGly m 8 (data not shown).

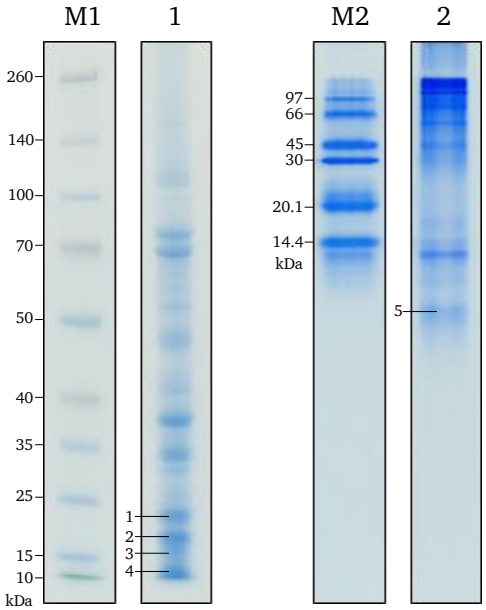


Figure 34: SDS-PAGE and native PAGE of soybean extract for MS analysis.

Lane 1, SDS-PAGE of soybean extract under reducing conditions; lane 2, native/non-denaturing PAGE of soybean extract; M1, Spectra™ Multicolor Broad Range Protein Ladder used in SDS-PAGE; M2, low-molecular weight marker used in native PAGE.

Table 35: Identification of Gly m 8 in soybean extract.

Band	Acc. No. UP	Description	S	P	C	E
1	P19594	Gly m 8 2S Albumin *Glycine max*	4954	3	29.7	0.8
2	P19594	Gly m 8 2S Albumin *Glycine max*	12467	5	35.4	1.8
3	P19594	Gly m 8 2S Albumin *Glycine max*	42510	7	43.7	2.0
4	P19594	Gly m 8 2S Albumin *Glycine max*	15557	10	37.3	2.6
5	P19594	Gly m 8 2S Albumin *Glycine max*	1975	7	36.1	5.0

Acc.No. UP, accession number of Uniprot; S, PLGS protein score; P, number of identified peptides; C, protein sequence coverage %; E, precursor RMS mass error [ppm].

Subsequently, all patients were analyzed for their serum IgE binding to peptides of Gly m 8 and Gly m 5.03. Since some studies highlighted the high diagnostic value of sIgE to Gly m 8, all soybean patients were individually investigated for their serum IgE binding to peptides of Gly m 8, despite no relevant IgE binding to rGly m 8 and nGly m 8 could be identified so far.

Full-length Gly m 8 was represented by 32 peptides (see appendix Table A51). Visual evaluation of Gly m 8 microarrays revealed no serum IgE binding to peptides of Gly m 8 of all investigated soybean-allergic and tolerant children (see appendix Figure A21). Therefore, no Z-score calculation was performed. This finding was in accordance with the immunoblot analyses, where also no soybean-allergic patient showed a serum IgE binding to rGly m 8 and nGly m 8. Hence, in this small study group of soybean-allergic children, Gly m 8 was neither on the protein nor on the peptide level an allergen. Similarly, soybean-sensitized but tolerant patients showed no and no relevant IgE binding to rGly m 8 and nGly m 8, respectively. Thus, Gly m 8 is not a relevant protein with sensitizing properties in this study group of soybean-allergic and sensitized but tolerant children.

Some serum IgE binding was detected to peptides of Gly m 5.03. Thus, the results of this multipeptide microarray analysis will be described in more detail.

Figure 35 shows the spotting layout of the Gly m 5.03 multipeptide microarray, on which both isoforms, Gly m 5.0301 and Gly m 5.0302, of the subunit Gly m 5.03 were considered. Gly m 5.0302 was the sequence of the recombinant protein investigated in the immunoblot analysis. Figure 36 illustrates the sequence alignment of both isoforms and shows the two amino acid residue substitutions (Figure 36, red) from phenylalanine to leucine in Gly m 5.0301 compared to Gly m 5.0302. Similar to the peanut project, peptides shared by both isoforms were spotted once (half dark blue, half light blue spots in Figure 35A). Detailed peptide sequence information is presented in the appendix, in Table A50.

After spotting, staining with Coomassie confirmed consistent spotting of all peptide solutions on array slides (Figure 35B). As an example, the immunodetection using serum of soybean-allergic patient 2 is shown (Figure 35C). Images of the IgE immunodetections of all patients are shown in the appendix, in Figure A20.

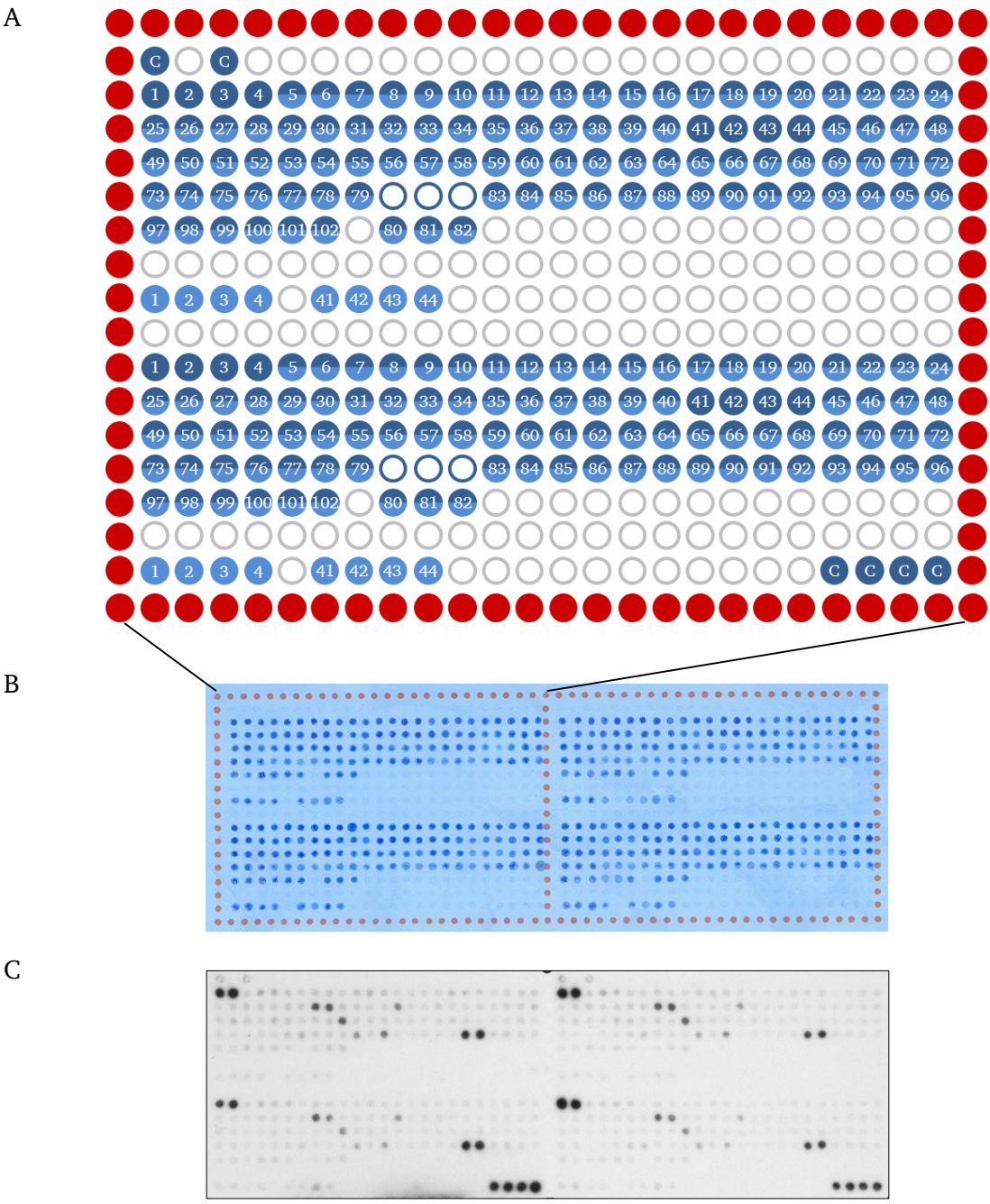


Figure 35: Spotting layout, Coomassie-staining and IgE immunodetection of Celluspot™ multipeptide microarray displaying Gly m 5.03.

(A) Spotting layout of Gly m 5.03 multipeptide microarray. For simplification, only the left segment of the multipeptide microarray is shown. Light and dark blue spots represent unique peptides either found in Gly m 5.0301 or Gly m 5.0302, respectively. Half dark blue, half light blue spots represent peptides shared by both isoforms. In general, both Gly m 5.03 isoforms are covered by 102 peptides. Biotinylated control peptides, abbreviated by a "C", were spotted in duplicate in different dilutions (top left and bottom right on each array element) and were used as position markers. Gray empty spots represent blank spots composed of peptide printing buffer (DMSO). Blue empty spots represent internal control peptides not relevant for peptide analysis. (B) Successful spotting was verified by staining with Coomassie Brilliant Blue G250. (C) IgE immunodetection using serum of a soybean-allergic child (patient 2; 30 sec exposure).

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Gly m 5.0301  1    LKVREDENNPFYLRSSNSFQTLFENQNGRIRLLQRFNKRSPQLENLRDYRIVQFQSKPNT  60
Gly m 5.0302  1    LKVREDENNPFYFRSSNSFQTLFENQNGRIRLLQRFNKRSPQLENLRDYRIVQFQSKPNT  60

Gly m 5.0301  61   ILLPHHADADFLLFVLSGRAILTLVNNDDRDSYNLHPGDAQRIPAGTTYYLVNPHDHQNL  120
Gly m 5.0302  61   ILLPHHADADFLLFVLSGRAILTLVNNDDRDSYNLHPGDAQRIPAGTTYYLVNPHDHQNL  120

Gly m 5.0301  121  KIIKLAIPVNKPGRYDDFFLSSTQAQQSYLQGFSHNILETSFHSEFEEINRVLLGEEEEQ  180
Gly m 5.0302  121  KIIKLAIPVNKPGRYDDFFLSSTQAQQSYLQGFSHNILETSFHSEFEEINRVLFGEEEEQ  180

Gly m 5.0301  181  RQQEGVIVELSKEQIRQLSRRAKSSSRKTISSEDEPFNLRSRNPIYSNNFGKFFEITPEK  240
Gly m 5.0302  181  RQQEGVIVELSKEQIRQLSRRAKSSSRKTISSEDEPFNLRSRNPIYSNNFGKFFEITPEK  240

Gly m 5.0301  241  NPQLRDLDIFLSSVDINEGALLLPHFNSKAIVILVINEGDANIELVGIKEQQQKQKQEEE  300
Gly m 5.0302  241  NPQLRDLDIFLSSVDINEGALLLPHFNSKAIVILVINEGDANIELVGIKEQQQKQKQEEE  300

Gly m 5.0301  301  PLEVQRYRAELSEDDVFVIPAAYPFVVNATSNLNFLAFGINAENNQRNFLAGEKDNVVRQ  360
Gly m 5.0302  301  PLEVQRYRAELSEDDVFVIPAAYPFVVNATSNLNFLAFGINAENNQRNFLAGEKDNVVRQ  360

Gly m 5.0301  361  IERQVQELAFPGSAQDVERLLKKQRESYFVDAQPQQKEEGSKGRKGPFPSILGALY  416
Gly m 5.0302  361  IERQVQELAFPGSAQDVERLLKKQRESYFVDAQPQQKEEGSKGRKGPFPSILGALY  416
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Figure 36: Sequence alignment of Gly m 5.0301 and Gly m 5.0302.

Mature full-length sequences of Gly m 5.0301 (PDB sequence 1IPJ) and Gly m 5.0302 (PDB sequence 1IPK) were aligned using BLAST® (NCBI, Bethesda MD, USA). Differences in amino acid sequence are shown in red.

Initial Z-score calculation resulted for some patients in high Z-scores after control subtraction, and consequently incomprehensible positive IgE-binding peptides. As an example, the immunodetection of patient 7 is shown in Figure 37. Depending on the analyzed Gly m 5.03 isoform, Z-score calculation resulted for this patient in at least 80 IgE-binding peptides with Z-score > 2. This result was not in accordance with the image of the immunodetection as visually no serum IgE binding to any of the peptides, except for one peptide (peptide no. 6), could be identified for this patient. A possible explanation for this might be the use of just one control serum (serum N) due to low serum availability in microarray analysis instead of multiple control sera as used in the peanut or pea project. Therefore, no detailed Z-score calculation was performed.

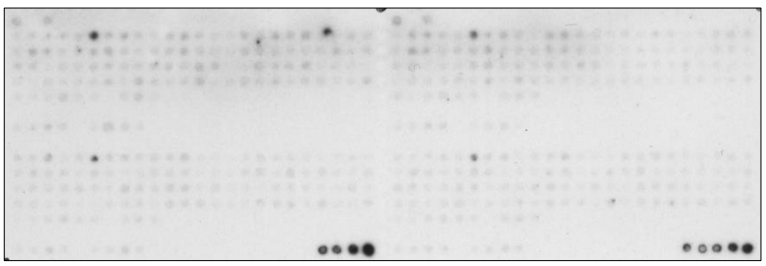

Figure 37: IgE immunodetection using serum of soybean-tolerant patient 7 after 30 sec exposure.

Visually, 2/6 soybean-allergic children (patients 1 and 2) showed a serum IgE binding to peptides of Gly m 5.03 (see appendix Figure A20). However, IgE binding was relatively weak and no significant differences could be observed with soybean-tolerant children, some of whom also showed IgE binding.

The limited number of soybean-allergic subjects, and in addition the limited serum IgE binding to only a few synthetic peptides of Gly m 5.03, did not allow for further analysis with regard to the aims of this project, i.e. to identify peptides as candidate markers for soybean allergy.

These observations highlighted the need to include more soybean-allergic patients in future analysis of IgE binding to Gly m 5.03 peptides.

5 Discussion

After a detailed analysis of the medical history, the anamnesis, the status quo in allergy diagnosis is the *in vitro* measurement of allergen-specific IgE (sIgE) in the blood, that is bound to allergens in total protein extracts or to single allergen components (Boyce et al. 2010; Renz et al. 2018; Yu et al. 2016). Nonetheless, with a few exceptions, the determination of the sIgE to extracts or extract components just predicts a sensitization rather than the clinical phenotype. Currently, the most specific verification is the double-blind, placebo-controlled food challenge (DBPCFC), but it is difficult to do on a routine basis, and also potentially risky for the patient. Thus, a simple and more specific *in vitro* test would be desirable.

Once an allergy is diagnosed, immunotherapy would be the first choice to modulate the immune system in a causative way to tolerate the allergen. Several allergen-specific therapeutic approaches using, for example, modified allergens or extracts are under investigation but for food allergy, no safe causative immunotherapy is currently available for humans (Cook and Burks 2018; Nowak-Węgrzyn and Sampson 2011). The detailed molecular knowledge of allergens and their interaction with the immune system may allow the design of therapeutic allergen variants with optimized characteristics, including reduced side effects.

In allergy diagnosis as well as in allergen-specific immunotherapy, allergen-derived peptides can potentially increase diagnostic accuracy and therapeutic efficacy as reported by several studies on humans and mice (Cook and Burks 2018; Lin et al. 2012; Simms et al. 2016).

In this PhD project, linear peptides/epitopes were investigated for their properties to bind serum IgE and whether this specific IgE binding can serve as a starting point towards the development of novel approaches for the *in vitro* allergy diagnosis and the design of novel therapeutic reagents, respectively. This PhD study focused on 2S albumin and 7S globulin storage proteins from peanut, pea and soybean, all of which are known allergenic legumes. Further, especially 2S and 7S storage proteins of legumes have been reported as being of high allergenic importance, especially in children (Beyer et al. 2015; Holzhauser et al. 2009; Ito et al. 2011; van Erp et al. 2017).

First, the status quo of specific sensitization, meaning the serum IgE binding to individual allergen components, was determined for each individual legume-allergic and legume-sensitized but clinically tolerant child. By doing so, the relevance of the single allergen components in each legume study population was determined. For allergen component testing and for subsequent IgE immunoblot and microarray inhibition experiments, 2S albumins and 7S globulins from the three investigated legumes were heterologously expressed, purified and

characterized. In addition, recombinant full-length proteins served as comparators for peptide analyses.

5.1 Generation of recombinant proteins

All recombinant 2S and 7S proteins were initially aimed to be expressed in *Pichia pastoris*, because yeast as eukaryote has been described to enable post-translational modifications like folding and disulfide bond formation (Cregg et al. 2000; Daly and Hearn 2005). These properties are especially advantageous if the recombinant proteins, such as the 2S albumins, are stabilized by disulfide bonds. In addition, cloning of the gene of interest into an expression vector containing the α-factor signal sequence enables the secretion of the recombinant protein and a simple non-denaturing purification. For better comparability of 2S and 7S proteins, 7S globulins should also be expressed using *Pichia pastoris*.

Despite a successful integration of all genes of interest into the yeast genome, only the 2S albumins were successfully expressed as full-length proteins using *Pichia pastoris*. Apart from pea PA2, all 2S albumins showed comparable physicochemical characteristics with a large portion of an α-helical-type secondary structure and hydrodynamic radii between 2.4 and 3.0 nm. In contrast, 7S globulins showed, in spite of their high nucleotide sequence identity (~70%), a high level of heterogeneity regarding their expression and/or secretion as full-length proteins. In contrast to soybean Gly m 5.03, peanut Ara h 1 and pea Pis s 1 could not be expressed as full-length proteins. The lack of expression of Ara h 1 in *Pichia pastoris* could have several causes. Some studies reported an influence of the adenine and thymine (AT) content on the transcription termination and recommended an AT content of 30-55% for proper transcription (Sreekrishna et al. 1997). However, all cDNA sequences were purchased as codon optimized sequences for the expression in *Pichia pastoris* and the AT content of Ara h 1 (54%) was identical to Pis s 1 (55%) and Gly m 5.03 (55%). In addition, no conspicuous AT-rich nucleotide stretches, such as ATTATTTTATAAA, could be found in the sequence of Ara h 1, excluding the AT content as possible explanation. Moreover, the expression of a full-length pea rPis s 1 failed. Instead, two proteolytic N- and C-terminal fragments of approximately 20 and 27 kDa were obtained. Despite several optimization strategies, the expression of rPis s 1 as full-length protein failed giving rise to the hypothesis that rPis s 1 is potentially cleaved intracellularly in *Pichia pastoris* into its natural subunits as described in the literature (Gatehouse et al. 1982; Sanchez-Monge et al. 2004). Taking into account the potential cleavage site of rPis s 1 (K168 and R187), an analysis of the N-terminal fragment revealed that the 20 kDa fragment could potentially correspond in molecular weight and amino acid sequence to the α-subunit (19 kDa) described by Gatehouse *et al.* (Gatehouse et al. 1982). In addition, the C-

terminal 27 kDa fragment could potentially correspond to the 32 kDa subunit composed of β + γ subunit described by Sanchez-Monge *et al.* (Sanchez-Monge et al. 2004). These findings may indicate a possible post-translational subunit-specific proteolysis of rPis s 1 in *Pichia pastoris*. Other studies also reported post-translational subunit-specific processing. A study from Lin and co-workers reported a post-translational proteolysis of soybean rGly m 8 subunits expressed in *Pichia pastoris* strain GS115. The authors showed that *Pichia pastoris* enabled proteolytic processing of rGly m 8 subunits within the same loop region as nGly m 8 but at different specific sites (Lin et al. 2004). However, this finding could not be confirmed and reproduced within this PhD project using *Pichia pastoris* strain X-33, because rGly m 8 was expressed as full-length proprotein without subunit processing.

Nevertheless, to obtain full-length rPis s 1, the expression system was changed to *Escherichia coli*. This resulted in full-length rPis s 1 that showed good accordance in its physicochemical characteristics with rGly m 5.03 expressed in *Pichia pastoris*. Peanut Ara h 1 used in this PhD project was also an *E. coli*-expressed recombinant protein that was kindly provided by Dr. Jonas Lidholm (Thermo Fisher Scientific).

Although the expression of 2S albumins and rGly m 5.03 was possible in *Pichia pastoris*, it must be noted that *Pichia pastoris* as expression system does not guarantee a successful protein expression even of homologous proteins. In addition, the expression in *Pichia pastoris* far took more time and resulted in lower protein yields when compared to *Escherichia coli* (0.07-2.3 mg vs. 8.5 mg).

5.2 Knowledge of specific IgE-binding sites of allergens for the development of novel therapeutic reagents

The knowledge of relevant allergens and their IgE-binding sites, i.e. epitopes, opens up new strategies for the rational design of novel therapeutic reagents with improved characteristics. For example, a reduced IgE-binding capacity by a rational design of therapeutic peptides or modified full-length allergens may reduce undesired side effects in immunotherapy, such as adverse events. Since the focus of this PhD project was more on allergy diagnosis, the therapeutic benefit of this project will be briefly described below.

Due to the very limited number of soybean-allergic patients included in the soybean project, no conclusions can be drawn. However, additional knowledge was gained for peanut and pea allergens that should be considered when developing novel therapeutic reagents.

Common therapeutic approaches comprise the use of hypoallergens generated by site-directed mutagenesis or chemical methods like reduction and alkylation (r/a) with the primary focus on destroying the folding of the allergen (Kahlert et al. 2008; Zuidmeer-Jongejan et al. 2012).

Here, the IgE-binding capacity should be reduced while maintaining the T-cell immunogenicity. This approach appears promising, however, based on the results on Ara h 2, the most relevant peanut allergen in this study population, merely destroying the protein structure may not be enough in order to create a safe immunotherapeutic molecule in this study population. Comparable to the results published by Bernard and co-workers, a strong remaining IgE binding to r/a nAra h 2 in peanut extract was observed (Bernard et al. 2015). A remarkable number of peanut-allergic children (70%) still showed an IgE binding to r/a nAra h 2, albeit partly to a lesser extent than to native nAra h 2. However, even for 50% (12/23) of the peanut-allergic children a significant IgE binding could be detected highlighting the presence of major linear IgE-binding epitopes on r/a nAra h 2. In addition, multi-peptide microarray data of Ara h 2 showed a strong IgE-binding capacity of peptides containing the linear DPYSPS motif, which was strongly increased when the second proline was hydroxylated. The hydroxylated motif is found in natural Ara h 2. The importance of proline hydroxylation for the induction of mast cell degranulation was further demonstrated by the fact that the 27-mer peptide without P^{OH} was binding serum IgE but, compared to the 27-mer with P^{OH}, could not induce a specific mediator release (see Figure 19A and 19B). In addition, Figure 19B shows that the reduction and alkylation of peanut extract led to a 10-fold decrease in its allergenic potency. Even though this appears to be a strong decrease of allergenicity, a 10-fold increase in dose would already result in the same mediator release. Considering the amount of Ara h 1 in peanut extract, Ara h 1 contributed only in part to the release of r/a peanut extract. In contrast, the 27-mer peptide with P^{OH} itself had a high allergenic potency, 10-fold higher than r/a peanut extract. Considering the amount of Ara h 2 and Ara h 1 in peanut total protein extract, and considering the molar ratio of the 27-mer peptide in Ara h 2 (~1:5), the peptide in r/a peanut extract likely had an allergenic potency that was even higher than that of Ara h 1. The r/a rAra h 2.02 contained the biological inactive 27-mer peptide without P^{OH} and did not have any relevant allergenic potency suggesting the lack of a potent linear IgE epitope when prolines are not hydroxylated. Consequently, it appears that the 27-mer peptide with P^{OH} had a relevant contribution to the allergenicity of r/a peanut extract. In contrast, the contribution of Ara h 3 seemed to be negligible according to the immunoblot results, where Ara h 3 was localized between the molecular masses of Ara h 1 and Ara h 2.

The data published by Bernard *et al.* on the relevance and the allergenic potency of linear IgE epitopes of Ara h 2 containing hydroxylated proline residues could thus be confirmed with this peanut study population (Bernard et al. 2015).

In conclusion, these data highlight that linear IgE-binding epitopes containing the $DPYSP^{OH}S$ motif must be considered in the development of novel therapeutic approaches for peanut allergy

as otherwise patients might be at high risk of unintended side effects. Reduction and alkylation of peanut extract or nAra h 2 (demonstrated by the 27-mer peptide P^{OH}) may not be a safe therapeutic approach as both possessed the ability to induce mast cell degranulation to a relevant extent. Drawn on the results of this PhD project, one possibility to avoid side effects during allergen-specific immunotherapy would be the use of r/a rAra h 2 lacking immunodominant conformational and linear IgE-epitopes that are relevant for mast cell degranulation. Thus, the allergenic potency and the T-cell immunogenicity of r/a rAra h 2 should be investigated in further studies with more patients, prior to its development as an immunotherapeutic reagent.

A first important aspect in the development of molecular therapeutic approaches is the identification of the relevant allergen(s). With the identification of Pis s 1 as the most relevant major pea allergen in this pea-allergic study population, this PhD project makes a decisive contribution to that. In addition, as this project was focusing on IgE binding to peptides in approximation to linear epitopes, immunodominant linear IgE-binding peptides of Pis s 1, which were predominantly located at the C-terminal part, were identified. These linear structures were recognized by IgE of the majority of pea-allergic children. Peptides 28, 55, 71, 84, 91, 94-96 and 101 bound serum IgE of more than 60% of pea-allergic children and could be inhibited in their serum IgE binding by rPis s 1 verifying their specificity. Of the peptides mentioned, the highest median Z-score values (of allergic-patients showing a positive peptide IgE binding) ranging from Z-score 9 to 15 could be observed for peptides 84, 95-96 and 101. Once immunodominant IgE-binding peptides are identified, critical amino acids dominating the IgE binding have to be determined, e.g. via alanine-walk on the microarray or bioinformatic tools assigning peptide reactivity to the molecular protein surface (Dall'Antonia et al. 2014; Negi and Braun 2009; Stanley et al. 1997; Subbarayal et al. 2013). Finally, the knowledge of functional amino acids dominating the IgE binding can in turn be used for the design of hypoallergenic substitution variants of Pis s 1, as was shown for example by Ferreira and co-workers on Bet v 1 (Ferreira et al. 1998).

5.3 Relevance and diagnostic value of individual legume allergen components

Measuring the sIgE to individual allergen components is, besides measuring the extract-specific IgE, currently the status quo in common allergy diagnosis (Renz et al. 2018). Up to now, several studies demonstrated the diagnostic benefit of component testing over extract testing leading to an increase of component-resolved diagnostics (CRD) in routine allergy care (Canonica et al. 2013). As standard *in vitro* method, the allergen component-specific IgE in the blood is

measured by means of ImmunoCAP™ analysis. This requires prior knowledge of the relevant allergens. However, controversial or incomplete data exist, at least in part, about the relevance of 2S and 7S storage proteins from peanut, pea and soybean.

Therefore, the relevance and the diagnostic value of the investigated legume allergens should be determined in each legume study population.

Several peanut allergens and isoforms were identified and characterized in numerous studies. Previous studies demonstrated that sIgE to Ara h 2 showed, compared to peanut extract and other peanut allergens, the best accuracy in the prediction of peanut allergy in children and infants (Beyer et al. 2015; Dang et al. 2012; Keet et al. 2013; Klemans et al. 2013b; Nicolaou et al. 2011; van Erp et al. 2017). If described, AUCs ranging from 0.90 to 0.99 could be determined in the aforementioned studies using ROC curve analysis. However, sensitivity and specificity varied within these studies. At a cut-off of 0.35 kU_A/L Ara h 2 sIgE, sensitivity varied from 81% to 100% and specificity from 71% to 96% (Beyer et al. 2015; Dang et al. 2012; Keet et al. 2013; Klemans et al. 2013b; Nicolaou et al. 2011).

All mentioned studies were based on the measurement of the sIgE by means of the ImmunCAP™ or ImmunoCAP™ISAC method, which uses the Ara h 2 isoform Ara h 2.01 expressed in *E. coli* (personal communication Dr. Jonas Lidholm, Thermo Fisher Scientific, and http://www.phadia.com/en/Products/Allergy-testing-products/ImmunoCAP-Allergen-Information/Food-of-Plant-Origin/Allergen-Components/rAra-h-1-recombinant-Peanut/).

Only one study by Codreanu and co-workers used rAra h 2.02 coupled to the ImmunoCAP™ and reported in their French study population, that originated from two clinical centers, at a cut-off of 0.23 kU_A/L a specificity and a sensitivity of 96% and 93%, respectively (Codreanu et al. 2011).

However, no study has so far investigated the diagnostic value of both Ara h 2 isoforms within one pediatric study population originating from one clinical center.

Due to low serum availability, sIgE levels to the recombinant peanut allergens Ara h 1, Ara h 2.01 and Ara 2.02 could not be determined using ImmunoCAP™ but were densitometrically quantified by immunoblot analysis. Despite the limitations associated with a densitometric quantification by means of immunoblot analysis, such as e.g. lower dynamic range and sensitivity, a good correlation could be observed between rAra h 2.01 and rAra h 2.02 sIgE levels, determined by densitometry, and rAra h 2.01 sIgE levels, determined by ImmunoCAP™ analysis, in the range of 0.35 to 40 kU_A/L.

Based on the calculated LOD values for IgE binding to recombinant proteins in immunoblot analysis, this study showed that rAra h 2.02 was more frequently bound by serum IgE from peanut-allergic children in comparison to rAra h 2.01 and rAra h 1. This finding is in line with

other published studies showing a higher IgE-binding capacity of Ara h 2.02 in comparison to Ara h 2.01 (Bernard et al. 2015; Hales et al. 2004). This trend was further confirmed using ROC curve analysis that showed a higher diagnostic value of sIgE to rAra h 2.02 (AUC 0.86) in comparison to sIgE to rAra h 2.01 (AUC 0.75) and rAra h 1 (AUC 0.51) determined by densitometric immunoblot analysis. In addition, sIgE to peanut extract, determined using ImmunoCAP™, showed an AUC of 0.79. The observed rank order regarding the diagnostic value (Ara h 2.02 > peanut extract > Ara h 1) is also in agreement with other studies analyzing the diagnostic value of sIgE to peanut extract, rAra h 2.01 and rAra h 1 (Ebisawa et al. 2012; Eller and Bindslev-Jensen 2013; Klemans et al. 2013b). This shows that, although a different method was used for the determination of the sIgE levels and another strategy was used for ROC curve analysis of densitometrically quantified sIgE levels, tendencies similar to published studies using ImmunoCAP™ analysis were achieved in this study.

sIgE to rAra h 2.01 quantified by ImmunoCAP™ analysis showed an AUC of 1.00 and reached at a cut-off of 0.35 kU_A/L a sensitivity of 96% and a specificity of 92% in this peanut study population reflecting a perfect discriminative ability. In addition, this result showed that a representative cohort was included in this PhD project, as comparable results with other study populations were obtained (Beyer et al. 2015; Nicolaou et al. 2011). 22/23 (96%) peanut-allergic children showed a sIgE level to rAra h 2.01 of > 0.35 kU_A/L, whereas only 15/23 (65%) and 18/23 (78%) peanut-allergic children showed a positive serum IgE binding (> LOD) in immunoblot analysis to rAra h 2.01 and rAra h 2.02, respectively. However, as already mentioned, it has to be taken into account that the ImmunoCAP™ method is much more sensitive than the applied immunoblot analysis. In addition, analyzing both Ara h 2 isoforms in a comparable manner by means of immunoblot analysis, it could be clearly shown that Ara h 2.02 is the isoform with the higher IgE-binding capacity. This finding leads to the suggestion that the use of rAra h 2.02 instead of rAra h 2.01 in ImmunoCAP™ analysis might lead to an even higher measurement sensitivity.

Furthermore, using mediator release assay this study demonstrated that rAra h 2.02 is a major potent peanut allergen that elicits a mast cell degranulation almost identical to that of peanut extract. In addition, rAra h 2.02 showed an approximately 100-fold higher allergenic potency in comparison to rAra h 1, which is in line with studies by Blanc *et al.* and Palmer *et al.* both using for their *in vitro* mediator release assays purified allergens from whole peanut extract (Blanc et al. 2009; Palmer et al. 2005).

In conclusion, this study on recombinant peanut allergens showed that rAra h 2.02 is, compared to the two other self-generated proteins rAra h 2.01 and rAra h 1, the most relevant peanut allergen with regard to IgE-binding and mediator release capacity. Of these three recombinant

peanut proteins, rAra h 2.02 showed the highest diagnostic value in densitometric immunoblot analysis. These properties make it a target molecule for therapeutic and diagnostic approaches.

Although pea has been recognized as an emerging allergenic food (personal communication Prof. Dr. K. Beyer) that has already been described as the elicitor of severe allergic reactions (Lavine and Ben-Shoshan 2019; Wensing et al. 2003), detailed data on the relevance and the diagnostic value of pea allergens are still lacking. Three pea proteins (Pis s 1, Pis s 2 and Pis s 3) were registered as allergens in the WHO/IUIS database of allergens, two of which (Pis s 1 and Pis s 2) are described as potential major pea allergens (Sanchez-Monge et al. 2004). So far, no pea albumin is registered in the WHO/IUIS database as allergen. Only two studies by Vioque and co-workers focused on IgE binding to the 2S pea albumins PA1 and PA2. The authors reported a positive and negative serum IgE binding of ten chickpea-sensitive patients to PA2 and PA1, respectively. However, the relevance of these two studies is questionable since no conclusive results were available and individual data on IgE binding were mostly undisclosed (Vioque et al. 1998; Vioque et al. 1999).

The comparison of the serum IgE binding to rPis s 1, rPA1 and rPA2 by means of immunoblot analysis showed that rPis s 1 was, compared to both recombinant pea albumins, the most relevant pea allergen in this pea study population. The great majority (79%) of pea-allergic children showed serum IgE binding to rPis s 1, unequivocally confirming it for the first time as a major allergen based on individual patient sera testing. In contrast, no serum IgE binding could be detected to rPA1 and rPA2, except for rPA1 and one pea-allergic patient (patient no. 1). An altered serum IgE binding to rPA1 due to the expression as a proprotein containing two propeptides could be excluded, as natural PA1 was also found as proprotein version in whole pea extract and no IgE binding was detected in this low molecular weight region in immunoblot analysis.

The relevance of rPis s 1 was further demonstrated by the majority of serum IgE binding to pea extract being inhibited by rPis s 1. In addition, the inhibition experiment illustrated no additional major pea allergens in this pea study population. This result is in accordance with the study published by Sanchez-Monge and co-workers, who observed the strongest IgE binding of a serum pool composed of eighteen pea-allergic patients to Pis s 1 in pea extract. Also in line with the mentioned study, no relevant serum IgE binding of pea-allergic patients to low molecular weight proteins like 2S albumins or lipid-transfer proteins could be detected (Sanchez-Monge et al. 2004). This finding is in contrast to the presented data on peanut where the strongest serum IgE binding could be detected to the 2S albumin Ara h 2.

For the first time the allergenic potency of rPis s 1 was investigated and described. Using *in vitro* mediator release assay it could be shown that rPis s 1 was capable of eliciting mast cell degranulation. In addition, the shown data suggest that the majority of the biological activity of pea extract is caused by Pis s 1 making it a target molecule for therapeutic approaches. In addition, EC_{50} of rPis s 1 (0.11 ng/well) and pea extract (2.3 ng/well) differed around the factor 20 showing that Pis s 1 may account for approximately 5% of total pea protein, assuming that the great part of allergenic potency of pea total protein extract was related to Pis s 1. This estimated content is in agreement with other studies reporting that Pis s 1 comprises between 1% and 8% of the total protein in pea (Kroghsbo et al. 2011; Tzitzikas et al. 2006).

Currently, *in vitro* diagnosis of pea allergy is solely based on the ImmunoCAP™ analysis of specific IgE to pea total protein extract. However, using ROC curve analysis, this study showed that rPis s 1 (AUC 0.86) had a higher AUC and thus a better diagnostic value than pea total protein extract (AUC 0.81). As a consequence, the diagnostic performance of rPis s 1 should be further investigated using the ImmunoCAP™ method as it may lead to an even higher diagnostic sensitivity.

In conclusion, the presented data showed that Pis s 1 is the most relevant major allergen with a high diagnostic value in this pea study population. This 7S globulin can elicit mast cell degranulation and accounts for the majority of the allergenic potency of pea extract. In addition, it was shown that pea 2S albumins and other low molecular weight pea proteins appeared to be of no or only minor relevance in this pea study population.

Data on the relevance and the diagnostic value of soybean allergens are controversial. Depending on the respective study population and the study design, some authors reported an association of sIgE to Gly m 5 and/or Gly m 6 with the severity of allergic reactions and that sIgE to Gly m 5 and/or Gly m 6 might be a better predictor for soybean allergy compared to soybean extract (Holzhauser et al. 2009; Ito et al. 2011). Whereas other studies reported no predominant sensitization to Gly m 5 and Gly m 6 or reported that sIgE to Gly m 8 showed, compared to Gly m 5 and Gly m 6, the best diagnostic accuracy (Ebisawa et al. 2013; Fukutomi et al. 2012; Kattan and Sampson 2015; Vissers et al. 2011).

However, with regard to the results gained from this soybean study, the limited number of available and included soybean-allergic patients needs to be mentioned. The small sample size of allergic children hampered drawing general conclusions about the relevance and diagnostic value of Gly m 5.03 and Gly m 8. Nevertheless, tendencies were observed.

In this study, soybean-allergic and sensitized but tolerant children showed specific IgE binding to rGly m 5.03 (50% vs. 20%). A similar trend was reported by a study by Ito and co-workers

investigating sera from Japanese children. The study used comparable patient inclusion criteria and reported that 67% of soybean-allergic and 49% of sensitized but tolerant children showed an IgE reactivity to natural Gly m 5 (Ito et al. 2011). In addition, a study by Klemans *et al.* reported that 63% of soybean-allergic adults and 27% of soybean-tolerant adults showed a sensitization ($\geq$ 0.35 kU_A/L) to Gly m 5. However, tolerant adults were included in the Klemans study solely based on a negative food challenge, regardless of a positive sensitization to soybean extract (Klemans et al. 2013a). This was in contrast to this PhD study, which included tolerant patients based on a positive sensitization ($\geq$ 0.35 kU_A/L) to soybean extract.

No serum IgE binding to rGly m 8 could be observed in this soybean study population. This finding is in line with a study by Lin and co-workers, who reported that none of the 16 investigated sera from European soybean-allergic patients showed an IgE binding to recombinant and natural Gly m 8 purified from soybean extract (Lin et al. 2006). However, other studies reported a predominant role of sIgE to Gly m 8 in the prediction of soybean allergy. Using sera from either soybean-allergic children from Japan, the US or from soybean-allergic adults from Europe, the authors reported a sensitization to natural Gly m 8 with an AUC between 0.75 and 0.82 as the best diagnostic marker for clinically relevant soybean allergy (Ebisawa et al. 2013; Kattan and Sampson 2015; Klemans et al. 2013a). This could not be confirmed in this study population. To exclude that rGly m 8 expressed as proprotein showed a different IgE reactivity compared to the natural Gly m 8 purified from soybean extract, a native PAGE of soybean total protein extract was performed. The MS data suggested the presence of nGly m 8 as full-length protein without propeptide. However, it remains difficult to interpret why, under reducing conditions, full-length natural Gly m 8 could also be identified, because both Gly m 8 chains that are linked by disulfide bonds should be separated under reducing conditions (Ebisawa et al. 2013; Lin et al. 2004). Nonetheless, when using native PAGE of soybean total protein extract and subsequent IgE immunoblot analysis, no serum IgE binding could be detected to nGly m 8 in soybean-allergic patients. In addition, using SDS-PAGE under reducing conditions and IgE immunoblot analysis, the highest IgE reactivities could be attributed to 7S and 11S globulins. This is in accordance with a study by Ballmer-Weber and co-workers who showed strong serum IgE binding to 7S and 11S globulins (Ballmer-Weber et al. 2007).

The discrepancy in the relevance of Gly m 8 between this study, which was in line with the study by Lin *et al.*, and the above-mentioned studies may be due to different study populations investigated or different Gly m 8 preparations. Interestingly, all studies reporting a high diagnostic value of Gly m 8 were probably based on the same non-commercial Gly m 8

ImmunoCAP™ material (Ebisawa et al. 2013; Kattan and Sampson 2015; Klemans et al. 2013a).

In conclusion, the data discussed in this chapter show that legumes, despite their taxonomic relation, behave very differently with regard to the relevance of their respective allergens. In each investigated legume allergy, a specific relevance of the analyzed 2S and 7S storage proteins could be observed. In peanut, Ara h 1 and Ara h 2 could be identified as relevant allergens. However, based on immunoblot analysis, the 2S albumin Ara h 2, in particular the isoform Ara h 2.02, was the most relevant allergen with the highest diagnostic value in this peanut study population. In contrast, such relevance of 2S albumins could not be detected in pea and soybean, which excludes 2S albumins as major allergens in both investigated study populations. In pea, the 7S globulin Pis s 1 was identified as the most relevant allergen in this study. A similar trend seemed to be observed for Gly m 5.03 and soybean, but due to the limited number of soybean-allergic patients included in this study, no further conclusions can be drawn.

5.4 Diagnostic potential of IgE-binding peptides of major legume allergens

Despite the advantages of a component-resolved diagnosis, studies on allergic and tolerant patients have also shown that there is often an overlap in the component sIgE level between both groups (Lin et al. 2012). Overlaps in the component sIgE levels can lead to misclassifications and demonstrate that there are still patients that need to undergo oral food challenge to determine their clinical phenotype.

Compared to extract- and component-resolved diagnosis, several studies suggested an additional diagnostic value of IgE-binding epitopes. Using Ara h 1-, Ara h 2- and Ara h 3-derived peptides, Beyer *et al.* and Shreffler *et al.* reported a correlation between serum IgE binding to specific peptides and clinical reactivity as well as between IgE epitope diversity and clinical severity (Beyer et al. 2003; Shreffler et al. 2004). In addition, another more recent study from Lin and co-workers, who analyzed the same peanut allergens, identified peptide biomarkers predicting peanut allergy with high accuracy by means of a machine learning method (Lin et al. 2012).

In addition to the potential to improve allergy diagnosis, peptide-based microarrays have further advantages such as, for example, low serum consumption and the potential to investigate multiple peptides of different allergens simultaneously. Furthermore, the synthesis of peptides and their quality control is straightforward in comparison to the generation of full-length proteins or the consistency control of protein extracts.

To assess whether peptides have the potential to improve allergy diagnosis, peptides representing all examined legume proteins were synthesized and analyzed for their serum IgE binding. By comparing IgE binding of sera from legume-allergic with sera from legume-sensitized but tolerant children, candidate diagnostic peptides were identified and investigated for their potential to improve the accuracy of the *in vitro* allergy diagnosis.
Initially, the quality of the in-house synthesized peptides was verified. This was done by comparing in-house synthesized overlapping peptides comprising Ara h 2 for their IgE binding with the same peptides purchased from Intavis AG (peptide specialist). Using a PEI-internal control serum, identical IgE-binding peptides confirmed the quality of the in-house synthesized peptides (data not shown) and justified their use in microarray analyses.

Multiple immunodominant IgE epitopes have been described for Ara h 1 and Ara h 2 (Burks et al. 1997; Han et al. 2016; Shreffler et al. 2004; Stanley et al. 1997).
However, this PhD study is the first study so far that investigated the diagnostic value of synthetic peptides of Ara h 1 and Ara h 2 in direct comparison to the respective full-length proteins. In addition, this study examined the contribution of proline hydroxylation (Hyp) in the DPYS$\underline{P^{OH}}$S motif of Ara h 2, as present in natural Ara h 2, on the diagnostic accuracy.
For simplification, the full-length sequences of Ara h 2, which contain proline or hydroxyproline residues in the DPYS$\underline{P}$S motif, are abbreviated as “Ara h 2_P” or “Ara h 2_Hyp” in the following, respectively.
In line with the data of the recombinant allergens, a high diagnostic potential of Ara h 2 was also found at the peptide level. Compared to Ara h 1, only peanut-allergic children showed a serum IgE binding to Ara h 2-derived peptides, whereas Ara h 1-derived peptides were also recognized by peanut-tolerant patients. Furthermore, Ara h 1-derived peptides reached a maximum IgE-binding frequency of 43%, whereas Ara h 2_Hyp-derived peptides reached a maximum IgE-binding frequency of 70%. The finding that no Ara h 1-derived peptide was bound by serum IgE of more than 43% of peanut-allergic patients is in contrast to the initial study by Burks and co-workers, who reported serum IgE binding to individual peptides of more than 80% (Burks et al. 1997). However, for the identification of the immunodominant IgE-binding epitopes of Ara h 1, Burks and co-workers analyzed only ten patients individually. In addition, a further reason for the discrepancy between the study from Burks *et al.* and this study could be the difference in the study population with regard to patients’ age. The mean age in the Burks study was 25 years, whereas the mean age of peanut-allergic patients in this study was 4 years. In addition, Lin *et al.* reported IgE-binding frequencies of up to ~70% to individual

Ara h 1-derived peptides using sera of peanut-allergic children; however, they used a different more sensitive detection system (Lin et al. 2012).

On the other hand, the finding of this PhD study is in agreement with Shreffler *et al.*, who reported a serum IgE binding to individual peptides of Ara h 1 in less than 30% of the analyzed patients (age: 1.5-36 years). However, compared to this PhD study, in which peanut allergy was confirmed by food challenge in all patients, Shreffler *et al.* included patients with confirmed peanut allergy as well as patients with suspected peanut allergy. Suspicion of allergy was based exclusively on elevated peanut sIgE levels, but the history of peanut ingestion was unknown (Shreffler et al. 2004). In addition, a study by Beyer *et al.* also reported a lower IgE-binding frequency of peanut-allergic patients to immunodominant linear epitopes of Ara h 1 compared to epitopes of Ara h 2 and Ara h 3 (Beyer et al. 2003).

In this PhD study, three peptides of Ara h 1, peptide 17, 75 and 86, fulfilled the criteria for candidate diagnostic peptides, which were defined prior to analysis. A comparison of the three candidate diagnostic peptides with previously identified IgE-binding epitopes and immunodominant IgE epitopes showed that peptide 17 overlapped with the immunodominant epitope 4 and the IgE-binding epitope 5 identified by Burks *et al.* In addition, an overlap could also be detected between peptide 75 and IgE-binding epitope 12 of the same study (Burks et al. 1997). Due to peptides of different length and offset that were investigated by Burks and co-workers (10 AA, offset 8 AA) and in this PhD project (15 AA, offset 4 AA), IgE-binding epitopes described by Burks *et al.* overlap only partially with the identified candidate diagnostic peptides of this study. In addition, Beyer *et al.* analyzed sera from peanut-allergic and sensitized but tolerant patients for their IgE binding to immunodominant epitopes of Ara h 1, Ara h 2 and Ara h 3. The authors identified i.a. epitope 4 of Ara h 1, which overlapped with peptide 17 of this study, as useful for the prediction of clinically relevant peanut allergy (Beyer et al. 2003).

Peptide 86 was not identified as an important IgE-binding epitope in the initial study by Burks and co-workers, which was based on pooled sera (Burks et al. 1997). Only individual sera testing, as performed by Shreffler and co-workers, identified this sequence of Ara h 1 as important for serum IgE binding (Shreffler et al. 2004). However, it should be noted that both studies by Burks and Shreffler were done with the primary focus on identifying immunodominant IgE epitopes rather than on identifying candidate diagnostic peptides. Both studies focused on IgE epitopes that were recognized by the majority of peanut-allergic patients and did not include peanut-sensitized but tolerant patients for comparison. Furthermore, in contrast to this study, both studies did not perform inhibition experiments. However, since this was the case in this study, some of the published immunodominant IgE epitopes were not

identified as candidate diagnostic peptides because they did not fulfill the selection criteria established in this study.

In addition, using BLAST it could be identified that peptide 75 and 86 of Ara h 1 are part of conserved regions between Ara h 1 and Pis s 1 that may lead to IgE cross-reactivity. However, due to largely unknown co-sensitizations or co-allergies in this study population, further studies are necessary, to draw conclusions about possible IgE-binding regions which may result in serological and/or clinical cross-reactivity.

In contrast, no significant overlap was identified between these candidate peptides of Ara h 1 and Gly m 5.03.

To compare the diagnostic value of the three identified candidate diagnostic peptides of Ara h 1 with that of the full-length peanut allergens and of peanut extract, an ROC curve analysis was performed. This approach was similar to the study by Lin and co-workers, who also compared the diagnostic value of different analysis methods, such as multipeptide microarray and ImmunoCAP™, in one ROC curve analysis (Lin et al. 2012).

Even though different methods, like ImmunoCAP™, immunoblot and multipeptide microarray, were performed in this study, the ROC curve analysis clearly showed that the peptides of Ara h 1 as well as full-length rAra h 1 did not contribute to an improvement of the *in vitro* diagnosis of peanut allergy.

In line with the immunoblot analysis, serum IgE of peanut-sensitized but tolerant patients did not bind to any peptide of Ara h 2.01_P and Ara h 2.02_P. Even peptides of both Ara h 2 sequences containing hydroxyproline residues in the DPYS$\underline{P^{OH}}$S motif, Ara h 2.01_Hyp and Ara h 2.02_Hyp, did not bind serum IgE of tolerant patients. In contrast, the great majority of peanut-allergic patients showed serum IgE binding to peptides of Ara h 2. Moreover, in peanut-allergic patients, peptide 2 and peptides containing the DPYSPS motif could be identified as immunodominant IgE-binding epitopes in this study. This finding is in line with previous studies on Ara h 2-derived peptides (Han et al. 2016; Hansen et al. 2016; Stanley et al. 1997). Both, the initial study by Stanley and co-workers and the more recent study by Han and co-workers identified three peptides of Ara h 2.01 containing the immunodominant IgE epitopes. One of these three peptides overlapped with the immunodominant peptide 2 identified in this PhD study and the two others contained, in accordance with this study, the DPYSPS motif (Han et al. 2016; Stanley et al. 1997). In addition, Hansen *et al.* identified the same important IgE-binding peptides of the second isoform Ara h 2.02 (Hansen et al. 2016). However, all three previous studies reported also a strong IgE binding to peptides comprising the C-terminal part of Ara h 2.01 and Ara h 2.02. This is in contrast to this study where only 4-13% of peanut-allergic children showed an IgE binding to peptides comprising the C-terminal part of Ara h 2.

Reasons for this discrepancy may be that the initial study by Stanley *et al.* was predominantly based on pooled sera of adult patients and not on individual testing of serum samples of children (Stanley et al. 1997). Furthermore, Han *et al.* and Hansen *et al.* predominantly included patients with high peanut sIgE levels and adult patients, respectively (Han et al. 2016; Hansen et al. 2016). However, the influence of proline hydroxylation on the diagnostic value of Ara h 2-derived peptides has never been investigated on the peptide level using microarray analysis, although some studies reported a higher IgE-binding capacity and immunogenicity of linear IgE-epitopes of Ara h 2 due to hydroxylation (Bernard et al. 2015; Deak et al. 2017). In line with these studies, an increase in IgE-binding capacity could be observed in peptides containing hydroxylated compared to unhydroxylated proline residues. Proline hydroxylation led to an increase in the number of IgE-bound peptides, to higher signal intensities of IgE-bound peptides and to higher IgE-binding frequencies of peanut-allergic children. In addition, it could be shown that proline hydroxylation resulted in a higher sensitivity while maintaining the specificity, which makes this post-translational modification an interesting target for future diagnostic approaches. An increase in IgE-reactivity due to post-translational proline hydroxylation was also reported for Phl p 1, a major timothy grass pollen allergen (Petersen et al. 1998).

The stronger IgE-binding capacity of peptides containing hydroxyproline instead of proline is not surprising, as the hydroxyl (OH) group constitutes a polar functional group enabling the formation of an additional hydrogen bond with other functional groups. Hydrogen bonds are described by Steiner as important intermolecular interactions (Steiner 2002), which potentially explain why peptides containing hydroxyproline residues may favor serum IgE binding.

Among the legumes, especially peanut is associated with severe allergic reactions (Worm et al. 2014). As Ara h 2 is the only legume allergen containing hydroxylated proline residues one might speculate that the post-translational hydroxylation might be one reason for the high allergenicity of Ara h 2 and consequently of peanut.

Four peptide pairs of the analyzed Ara 2.01 and Ara h 2.02 sequences could be identified meeting the predefined selection criteria for candidate diagnostic peptides. Candidate peptides were part of the immunodominant IgE-binding epitopes and overlapped with previously identified immunodominant IgE-binding peptides (Han et al. 2016; Hansen et al. 2016; Stanley et al. 1997). ROC curve analysis showed that the two selected peptide pairs of Ara h 2.01_Hyp (peptide 2 and peptide 11) and of Ara h 2.02_Hyp (peptide 11 and peptide 15) had, with an AUC of 0.87-0.90, the best diagnostic accuracy of all identified candidate diagnostic peptides in this study population. Moreover, the two selected peptide pairs showed a comparable AUC with other studies that analyzed sIgE to rAra h 2.01 by means of ImmunoCAP™ (Beyer et al.

2015; Klemans et al. 2013b). Due to the consideration of hydroxyproline residues in this study, a sensitivity of 70% at a specificity of 100% could be achieved with each of these two candidate diagnostic peptide pairs of Ara h 2.01_Hyp or Ara h 2.02_Hyp. In contrast, other studies, that analyzed sIgE to rAra h 2.01, reported lower sensitivities (23%-43%) at a specificity of 100% (Dang et al. 2012; Keet et al. 2013; Klemans et al. 2013b).

Interestingly, Lin and co-workers used microarray data combined with bioinformatic methods and selected two peptides of Ara h 2.01 as key peptide biomarkers that showed an overlap with peptide 2 and peptide 11 identified in this study (Lin et al. 2012). Nevertheless, the authors did not consider proline hydroxylation in their study.

In conclusion, candidate diagnostic peptides could be identified of Ara h 1 and Ara h 2. Despite the fact that different methods were compared for their diagnostic accuracy in ROC curve analysis in this study, it was clearly shown that Ara h 1-derived peptides as well as full-length rAra h 1 showed, compared to rAra h 2 (rAra h 2.01 and rAra h 2.02) and derived peptides, the lowest diagnostic accuracy in this peanut study population. The two peptide pairs of Ara h 2.01_Hyp and Ara h 2.02_Hyp, investigated in microarray analysis, were comparably sensitive as the full-length rAra h 2.02, investigated in immunoblot analysis, and had a comparable diagnostic value. Furthermore, it was shown that proline hydroxylation had a positive impact on the diagnostic sensitivity and accuracy. With regard to proline hydroxylation, it should be emphasized that peptides have the advantage over recombinant proteins that such post-translational modifications can be considered. This advantage highlights their potential in the future diagnosis of peanut allergy.

As the microarray is less sensitive than the ImmunoCAP™, it would be interesting to couple the identified candidate diagnostic peptides to the ImmunoCAP™ and investigate their diagnostic sensitivity and specificity. In this study population, however, such advanced investigation was not feasible due to low serum availability.

To date, no study so far focused on the diagnostic value of single pea proteins and derived peptides. Pis s 1 could be identified in this pea study population as the most relevant major pea allergen having a diagnostic value better than pea extract. In the next step, peptides derived from Pis s 1 were investigated for their potential to further improve the *in vitro* diagnosis of pea allergy. Comparable to Ara h 1-derived peptides, pea-allergic as well as pea-sensitized but tolerant children showed a serum IgE binging to peptides of Pis s 1.

Pis s 1 peptides 28, 30, 55, 71, 84, 91, 94-96 and 101 bound IgE by the great majority of pea-allergic children (> 60%) and, thus, were identified as immunodominant IgE-binding peptides. Of these, the C-terminal peptides 84, 95, 96 and 101 showed the highest signal intensities. A

comparison using BLAST of these immunodominant IgE-binding peptides of Pis s 1 with the four immunodominant IgE-binding peptides of Ara h 1 described by Burks and co-workers revealed no overlap between these peptides. However, by using a serum pool composed of 15 peanut-allergic patients, Burks *et al.* identified additional IgE-binding epitopes of Ara h 1, which show a partial sequence overlap with the major IgE-binding peptides at the C-terminal end of Pis s 1. C-terminal peptides 91 and 94-96 share conserved amino acid residues with the previously identified epitopes 19-21 of Ara h 1 (Burks et al. 1997). In addition, immunodominant peptide 84 of Pis s 1 is part of a conserved region between Pis s 1 and Ara h 1. However, this region is not described in previous studies as an IgE-binding epitope of Ara h 1. Furthermore, Sun *et al.* predicted and characterized eleven major immunodominant linear IgE epitopes of Gly m 5.01 (77% sequence identity to Gly m 5.03) using five sera from soybean-allergic adults (Sun et al. 2013). Using BLAST, it could be observed that the immunodominant peptide 30 of Pis s 1 and peptide 9 of Gly m 5.01 belong to conserved regions. Furthermore, it could be determined that peptide 55 of Pis s 1 and peptides 11 and 12 of Gly m 5.01 also share a conserved region. In addition, immunodominant peptides 84, 91 and 94-96 of Pis s 1 are part of conserved regions between Pis s 1 and Gly m 5.01. In Gly m 5.01 a partial sequence of these regions is described by Sun *et al.* as being bound by serum IgE of 2/5 soybean-allergic patients (Sun et al. 2013).

If further verified, homologous IgE-binding epitopes on different allergens might explain serological or clinical cross-reactivity between the legumes, whereas divergent IgE-binding epitopes might explain divergent allergic phenotypes.

The selection of candidate diagnostic peptides specific for pea allergy resulted in eleven peptides distributed over the entire sequence of Pis s 1. In contrast to full-length rPis s 1, which was recognized by 79% of pea-allergic children, 93% showed a serum IgE binding to at least one of the eleven candidate diagnostic peptides. In addition to sensitivity, specificity could also be increased from 80% to 100% using candidate diagnostic peptides.

Candidate diagnostic peptides of Pis s 1 share no significant sequence identity with IgE epitopes of Ara h 1 described by Burks *et al.* and Shreffler *et al.* (Burks et al. 1997; Shreffler et al. 2004). Nevertheless, candidate diagnostic peptides 64, 93 and 100 are part of conserved regions between Pis s 1 and Ara h 1. However, these regions were not described in previous studies as IgE-binding epitopes of Ara h 1. In addition, no overlap could be detected between the eleven candidate diagnostic peptides of Pis s 1 and the three candidate diagnostic peptides of Ara h 1. A comparison using BLAST of the sequence of the candidate diagnostic peptides of Pis s 1 with the described IgE-binding epitopes of Gly m 5.01 by Sun and co-workers, revealed that peptide 3 and peptide 53 of Pis s 1 share conserved regions with peptide 1 and peptide 11 of Gly m 5.01,

respectively. Moreover, sequence homology could be observed between peptides 64, 74, 93 and 100 of Pis s 1 and regions of Gly m 5.01, which were not described as IgE-binding regions by Sun *et al.* (Sun et al. 2013).

The analysis using BLAST showed that conserved IgE-binding regions between the legumes exist that can influence an accurate diagnosis of legume allergy using full-length legume proteins and highlight the potential of the more refined epitope-resolved diagnosis compared to a component-resolved diagnosis.

To what extent these conserved IgE-binding regions lead to serological cross-reactivities, which may result in additional allergies, remains an open question, because co-sensitizations or co-allergies are largely unknown in this study population.

The high diagnostic potential of peptides could be further shown by ROC curve analysis comparing pea extract, rPis s 1 and peptides for their diagnostic accuracy. The eleven candidate diagnostic peptides showed of all three test variables with an AUC of 0.99 the best accuracy in diagnosing pea allergy. The candidate peptides reached, at a specificity of 100%, an excellent sensitivity of 93%, highlighting that peptides of Pis s 1 may improve the diagnosis of pea allergy. In this study population, the eleven candidate diagnostic peptides could be further narrowed while keeping the sensitivity. To what extent the relatively large number of candidate peptides can be narrowed in other study populations, has to be investigated in future prospective studies.

In conclusion, eleven peptides could be identified of the major pea allergen Pis s 1 that may, compared to pea extract and rPis s 1, improve the accuracy in the diagnosis of pea allergy. A limited number of these peptides (peptide 3 and 53) overlapped with described IgE-binding epitopes of Gly m 5.01. In addition, a total of four of the eleven peptides (peptides 64, 74, 93 and 100) belong to conserved regions, which, however were not described as IgE-epitopes of Ara h 1 and Gly m 5.01. These findings highlight the potential and the advantage of an epitope-resolved diagnosis compared to an extract or component-resolved diagnosis.

5.5 Concluding remarks

Legumes show, despite of their close taxonomic relation, significant differences with regard to the relevance of their 2S and 7S storage proteins. In peanut, the 2S albumin Ara h 2, especially the isoform Ara h 2.02, is the most relevant allergen showing the best diagnostic value of the investigated recombinant peanut allergens in immunoblot analysis. This finding is in contrast to pea and soybean, where 2S albumins could not be identified as relevant allergens. In pea, it could be shown that the 7S globulin Pis s 1 is the most relevant allergen. A similar tendency seems to be observed in soybean with the homologous allergen Gly m 5.03. However, the very

limited number of soybean-allergic patients included in this study does not allow to draw further conclusions.

In contrast to soybean, candidate diagnostic peptides could be identified for peanut and pea allergy. Two identified peptide pairs of Ara h 2.01_Hyp and Ara h 2.02_Hyp with AUC (0.87-0.90) showed a comparable diagnostic value as rAra h 2.02 (AUC 0.86) in immunoblot analysis. For pea allergy, eleven peptides (AUC 0.99) could be identified of Pis s 1 showing a diagnostic value better than that of pea extract (AUC 0.81) or rPis s 1 (AUC 0.86).

Analyzing the serum IgE binding on the epitope level instead of on the whole protein level seems to be a promising diagnostic approach highlighting that peptides may serve as additional or alternative reagents in the *in vitro* diagnosis of legume allergy. However, further prospective studies should verify if these peptides can be applied as biomarkers in clinical routine. In addition, coupling of the identified peptides to the more sensitive ImmunoCAP™ solid phase would be an additional interesting add-on experiment to investigate a potential improvement of the sensitivity of the candidate peptides.

Moreover, based on the gained data, more efficacious and safer therapeutic approaches can be deduced. Based on the mast cell degranulation that was triggered by the 27-mer peptide containing hydroxylated proline residues, it could be shown for peanut that linear IgE-binding epitopes must be considered in the development of safe therapeutic approaches, as otherwise patients might be at high risk of unintended side effects. In addition, identified immunodominant IgE-binding peptides of Pis s 1 may allow to generate hypoallergenic substitution variants in the near future.

In summary, the data presented in this thesis, may form a basis towards the development of novel diagnostic and therapeutic reagents in food allergy.

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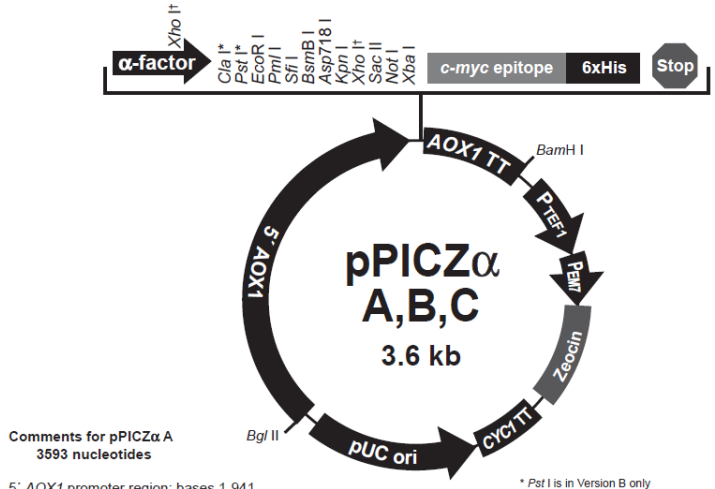
7 Appendix

7.1 Supporting material

7.1.1 Plasmids

A



B

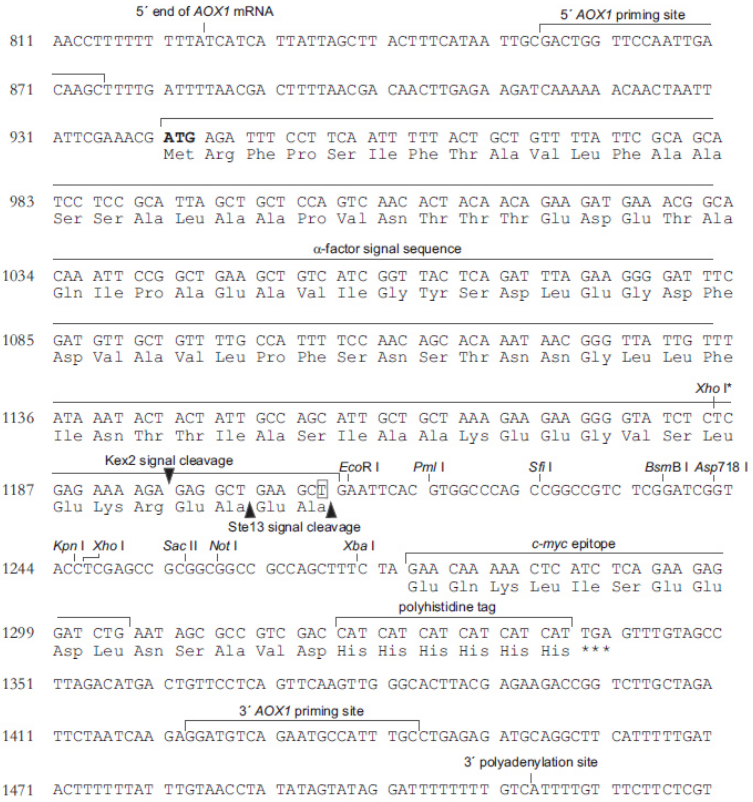
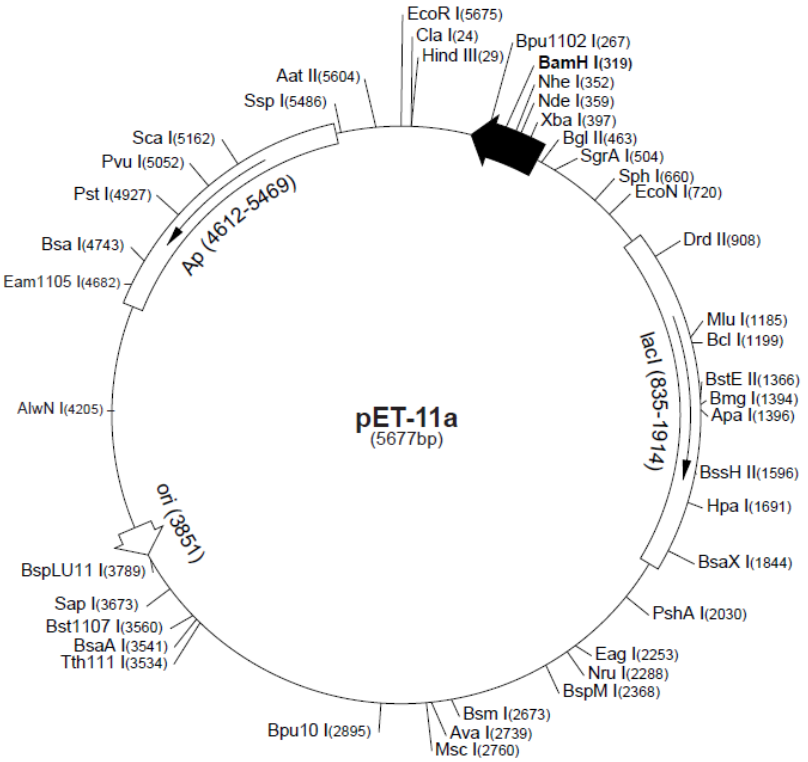


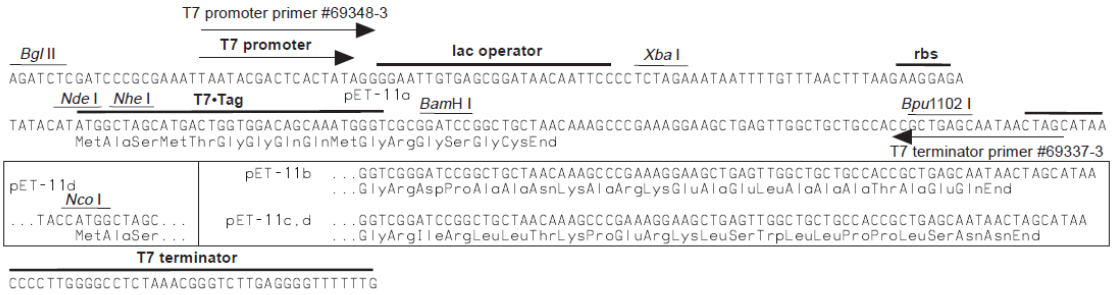
Figure A1: Vector map and multiple cloning site of pPICZαA.

(A) Vector map of pPICZαA. (B) Multiple cloning site of pPICZαA. Both images were taken from the user manual (Thermo Fisher Scientific, catalog number V195-20, publication number MAN0000035).

A



B



pET-11a-d cloning/expression region

Figure A2: Vector map and multiple cloning site of pET-11a.

(A) Vector map of pET-11a. (B) Multiple cloning site of pET-11a. Both images were taken from the user manual (Novagen, pET-11a-d Vectors, TB042 12/98).

7.1.2 Sequences of investigated recombinant proteins

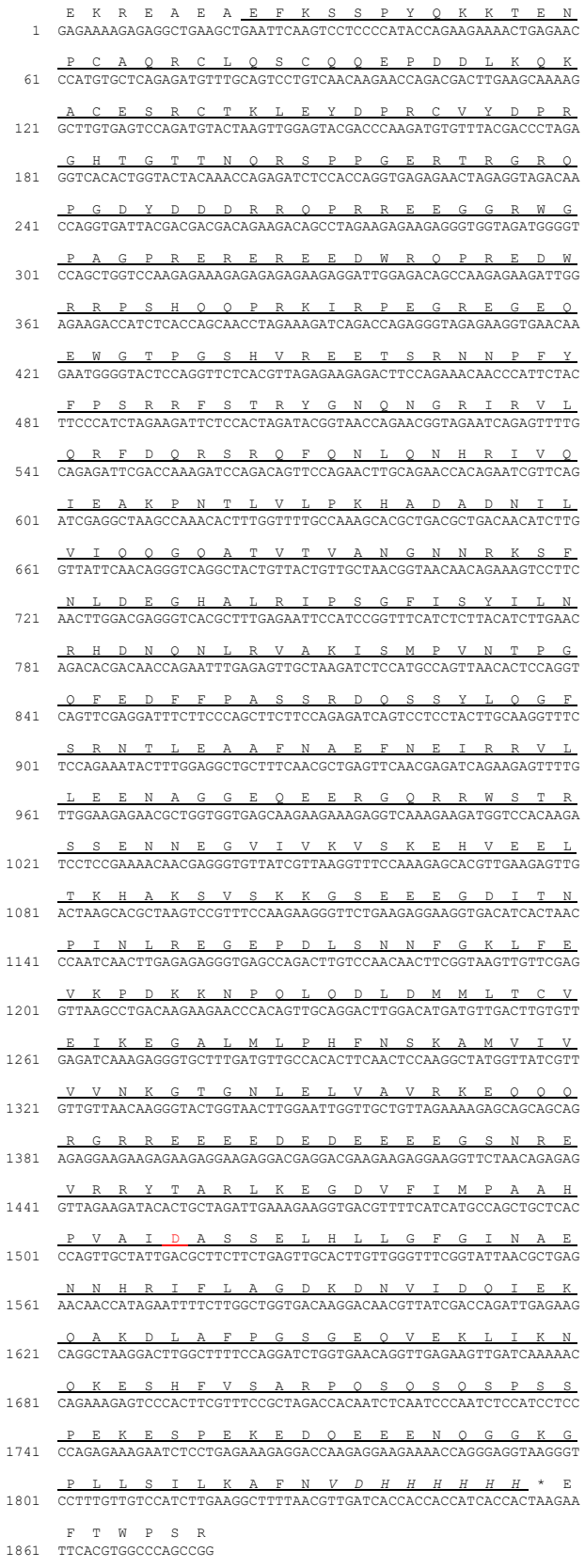


Figure A3: DNA fragment of rAra h 1 used for cloning into pPICZαA.

The amino acid sequence of rAra h 1 is underlined. rAra h 1 contained two additional N-terminal amino acids (EF) due to cloning into EcoRI restriction site. Linker (VD) and His_6-tag are written in italics. N496D is shown in red.

```
     E  K  R  E  A  E  A  E  F  R  Q  Q  W  E  L  Q  G  D  R  R
1    GAGAAAAGAGAGGCTGAAGCTGAATTCAGACAGCAGTGGGAATTGCAGGGTGACAGAAGA

     C  Q  S  Q  L  E  R  A  N  L  R  P  C  E  Q  H  L  M  Q  K
61   TGTCAATCCCAGTTGGAGAGAGCTAACTTGAGACCTTGTGAGCAGCACTTGATGCAAAAG

     I  Q  R  D  E  D  S  Y  E  R  D  P  Y  S  P  S  Q  D  P  Y
121  ATCCAGAGAGATGAGGACTCCTACGAGAGAGATCCATACTCTCCATCTCAAGACCCTTAC

     S  P  S  P  Y  D  R  R  G  A  G  S  S  Q  H  Q  E  R  C  C
181  TCCCCATCTCCATACGATAGAAGAGGTGCTGGTTCTTCCCAACACCAAGAGAGATGTTGT

     N  E  L  N  E  F  E  N  N  Q  R  C  M  C  E  A  L  Q  Q  I
241  AACGAGTTGAACGAGTTCGAGAACAACCAGAGATGTATGTGTGAGGCTTTGCAGCAGATC

     M  E  N  Q  S  D  R  L  Q  G  R  Q  Q  E  Q  Q  F  K  R  E
301  ATGGAAAACCAGTCCGACAGATTGCAAGGTAGACAACAAGAGCAGCAGTTCAAGAGAGAA

     L  R  N  L  P  Q  Q  C  G  L  R  A  P  Q  R  C  D  L  D  V
361  TTGAGAAACTTGCCACAGCAGTGTGGTTTGAGAGCTCCACAAAGATGTGACTTGGACGTT

     E  S  G  G  R  D  R  Y  V  D  H  H  H  H  H  H  *  E  F  T
421  GAATCTGGTGGTAGAGACAGATACGTTGATCACCATCATCACCACCACTAAGAATTCACG

     W  P  S  R
481  TGGCCCAGCCGG
```

Figure A4: DNA fragment of rAra h 2.01 used for cloning into pPICZαA.

The amino acid sequence of rAra h 2.01 is underlined. rAra h 2.01 contained two additional N-terminal amino acids (EF) due to cloning into EcoRI restriction site. Linker (VD) and His_6-tag are written in italics.

```
     E  K  R  E  A  E  A  E  F  R  Q  Q  W  E  L  Q  G  D  R  R
1    GAGAAAAGAGAGGCTGAAGCTGAATTCAGACAGCAGTGGGAATTGCAGGGTGACAGAAGA

     C  Q  S  Q  L  E  R  A  N  L  R  P  C  E  Q  H  L  M  Q  K
61   TGTCAATCCCAGTTGGAGAGAGCTAACTTGAGACCTTGTGAGCAGCACTTGATGCAAAAG

     I  Q  R  D  E  D  S  Y  G  R  D  P  Y  S  P  S  Q  D  P  Y
121  ATCCAGAGAGATGAGGACTCCTACGGTAGAGATCCATACTCTCCATCTCAAGACCCTTAC

     S  P  S  Q  D  P  D  R  R  D  P  Y  S  P  S  P  Y  D  R  R
181  TCCCCATCCCAAGATCCTGATAGAAGAGATCCTTATTCACCATCCCCATACGACAGAAGA

     G  A  G  S  S  Q  H  Q  E  R  C  C  N  E  L  N  E  F  E  N
241  GGTGCTGGTTCTTCTCAACACCAAGAGAGATGTTGTAACGAGTTGAACGAGTTCGAGAAC

     N  Q  R  C  M  C  E  A  L  Q  Q  I  M  E  N  Q  S  D  R  L
301  AACCAGAGATGTATGTGTGAGGCTTTGCAGCAGATCATGGAAAACCAGTCCGACAGATTG

     Q  G  R  Q  Q  E  Q  Q  F  K  R  E  L  R  N  L  P  Q  Q  C
361  CAAGGTAGACAACAAGAGCAGCAGTTCAAGAGAGAATTGAGAAACTTGCCACAGCAGTGT

     G  L  R  A  P  Q  R  C  D  L  E  V  E  S  G  G  R  D  R  Y
421  GGTTTGAGAGCTCCACAAAGATGTGACTTGGAAGTTGAGTCTGGTGGTAGAGACAGATAC

     V  D  H  H  H  H  H  H  *  E  F  T  W  P  S  R
481  GTTGATCATCACCATCACCACCACTAAGAATTCACGTGGCCCAGCCGG
```

Figure A5: DNA fragment of rAra h 2.02 used for cloning into pPICZαA.

The amino acid sequence of rAra h 2.02 is underlined. rAra h 2.02 contained two additional N-terminal amino acids (EF) due to cloning into EcoRI restriction site. Linker (VD) and His_6-tag are written in italics.

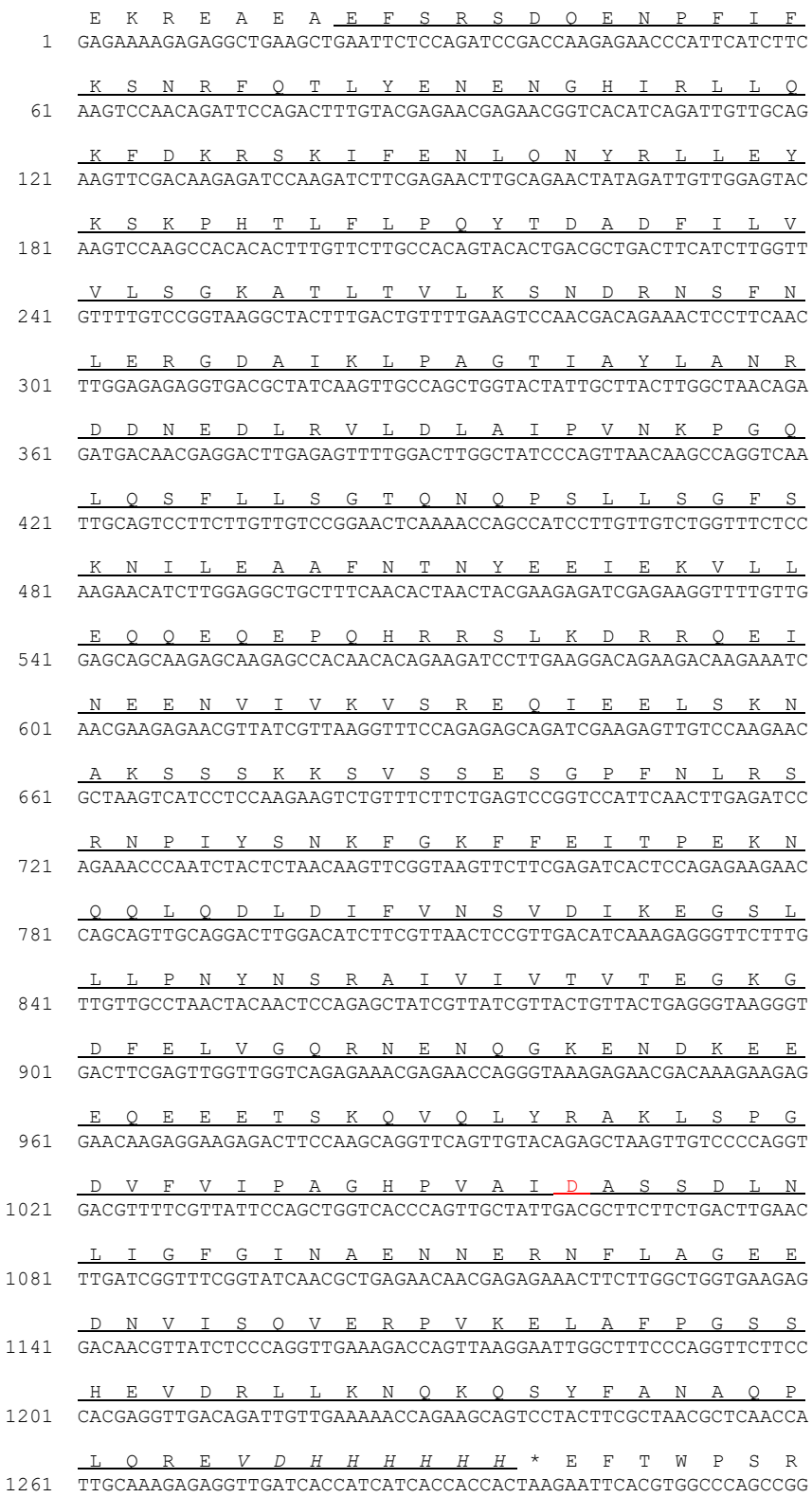


Figure A6: DNA fragment of rPis s 1 used for cloning into pPICZαA.

The amino acid sequence of rPis s 1 is underlined. rPis s 1 contained two additional N-terminal amino acids (EF) due to cloning into EcoRI restriction site. Linker (VD) and His_6-tag are written in italics. N345D is shown in red.

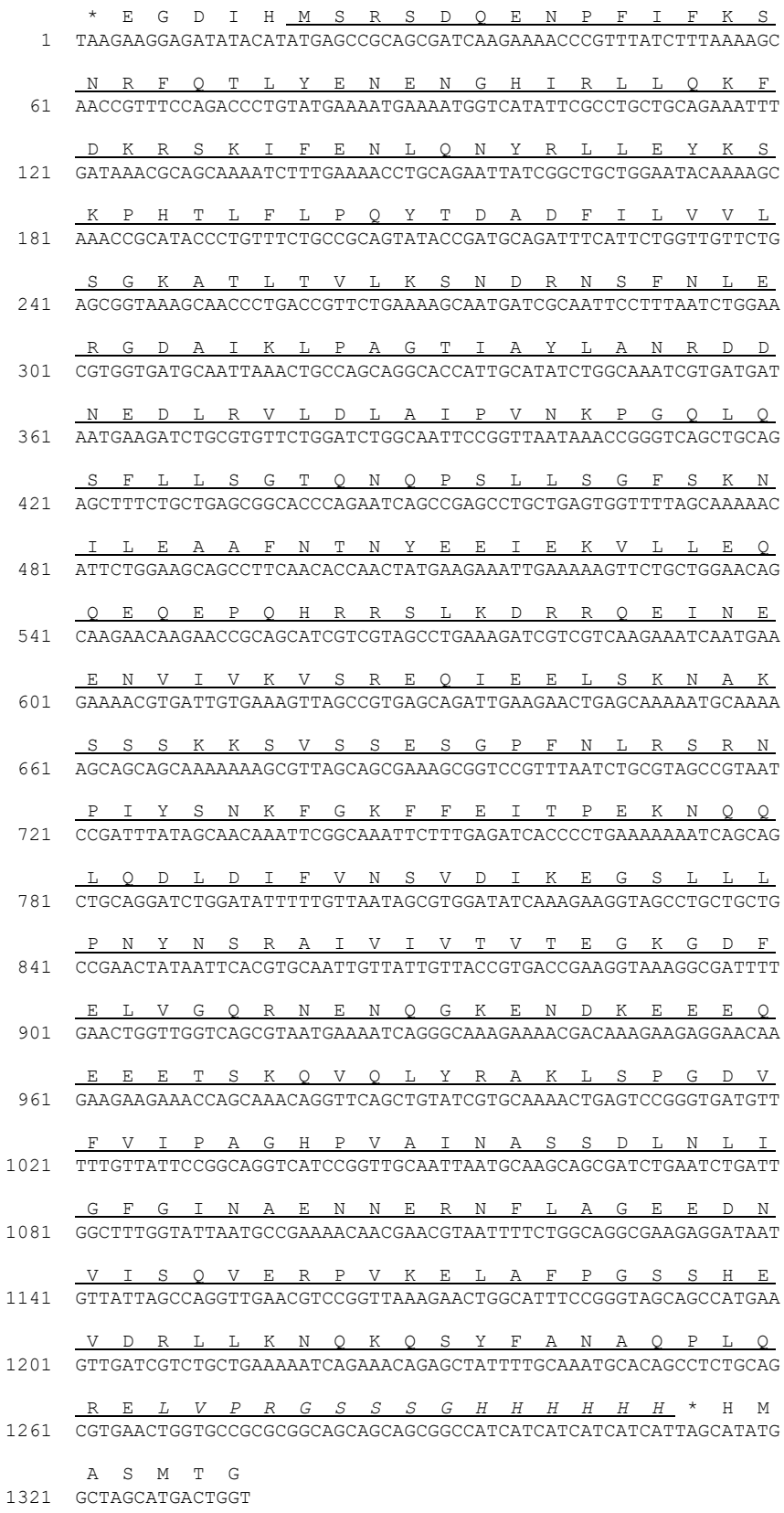


Figure A7: DNA fragment of rPis s 1 used for cloning into pET-11a.

The amino acid sequence of rPis s 1 is underlined. Thrombin cleavage site (LVPRGS), linker (SSG) and His_6-tag are written in italics.

```
     E  K  R  E  A  E  A  E  F  A  S  C  N  G  V  C  S  P  F  E
1    GAGAAAAGAGAGGCTGAAGCTGAATTCGCTTCCTGTAACGGTGTTTGTTCCCCATTCGAA

     M  P  P  C  G  S  S  A  C  R  C  I  P  V  G  L  V  V  G  Y
61   ATGCCACCATGTGGTTCTTCCGCTTGTAGATGTATCCCAGTTGGTTTGGTTGTTGGTTAC

     C  R  H  P  S  G  V  F  L  R  T  N  D  E  H  P  N  L  C  E
121  TGTAGACACCCATCCGGTGTTTTCTTGAGAACTAACGACGAGCACCCAAACTTGTGTGAA

     S  D  A  D  C  R  K  K  G  S  G  N  F  C  G  H  Y  P  N  P
181  TCTGACGCTGACTGTAGAAAGAAGGGTTCCGGTAACTTCTGTGGTCACTACCCAAACCCA

     D  I  E  Y  G  W  C  F  A  S  K  S  E  A  E  D  F  F  S  K
241  GACATTGAGTACGGTTGGTGTTTCGCTTCTAAGTCTGAGGCTGAGGACTTCTTCTCCAAA

     I  T  Q  K  D  L  L  K  S  V  S  T  A  V  D  H  H  H  H  H
301  ATCACTCAAAAGGACTTGTTGAAGTCCGTTTCCACTGCTGTTGATCACCATCATCACCAC

     H  *  E  F  T  W  P  S  R
361  CACTAAGAATTCACGTGGCCCAGCCGG
```

Figure A8: DNA fragment of rPA1 used for cloning into pPICZαA.

The amino acid sequence of rPA1 is underlined. rPA1 contained two additional N-terminal amino acids (EF) due to cloning into EcoRI restriction site. Linker (VD) and His_6-tag are written in italics. Propeptides are shown in blue.

```
     E  K  R  E  A  E  A  E  F  M  T  K  T  G  Y  I  N  A  A  F
1    GAGAAAAGAGAGGCTGAAGCTGAATTCATGACTAAGACTGGTTACATCAACGCTGCTTTC

     R  S  S  Q  N  N  E  A  Y  L  F  I  N  D  K  Y  V  L  L  D
61   AGATCCTCCCAAAACAACGAGGCTTACTTGTTCATCAACGACAAGTACGTTTTGTTGGAC

     Y  A  P  G  T  S  N  D  K  V  L  Y  G  P  T  P  V  R  D  G
121  TACGCTCCAGGTACTTCTAACGACAAGGTTTTGTACGGTCCAACTCCAGTTAGAGATGGT

     F  K  S  L  D  Q  T  V  F  G  S  Y  G  V  D  C  S  F  D  T
181  TTCAAGTCCTTGGACCAGACTGTTTTCGGTTCCTACGGTGTTGACTGTTCCTTCGATACT

     D  N  D  E  A  F  I  F  Y  E  K  F  C  A  L  I  D  Y  A  P
241  GACAACGACGAGGCTTTCATCTTCTACGAGAAGTTCTGTGCTTTGATCGATTACGCTCCA

     H  S  N  K  D  K  I  I  L  G  P  K  K  I  A  D  M  F  P  F
301  CACTCCAACAAGGACAAGATCATCTTGGGTCCAAAGAAAATCGCTGACATGTTCCCATTC

     F  E  G  T  V  F  E  N  G  I  D  A  A  Y  R  S  T  R  G  K
361  TTCGAGGGTACAGTTTTCGAGAACGGTATCGATGCTGCTTATAGATCCACTAGAGGTAAA

     E  V  Y  L  F  K  G  D  Q  Y  A  R  I  D  Y  E  T  N  S  M
421  GAGGTTTATTTGTTCAAGGGTGACCAGTACGCTAGAATCGACTACGAGACTAACTCCATG

     V  N  K  E  I  K  S  I  R  N  G  F  P  C  F  R  N  T  I  F
481  GTTAACAAAGAGATCAAGTCCATCAGAAACGGTTTCCCATGTTTCAGAAACACTATCTTC

     E  S  G  T  D  A  A  F  A  S  H  K  T  N  E  V  Y  F  F  K
541  GAGTCCGGTACTGATGCTGCTTTCGCTTCTCACAAGACTAACGAAGTTTACTTCTTTAAG

     G  D  Y  Y  A  R  V  T  V  T  P  G  A  T  D  D  Q  I  M  D
601  GGTGATTACTACGCTAGAGTTACTGTTACTCCAGGTGCTACTGACGACCAGATTATGGAC

     G  V  R  K  T  L  D  Y  W  P  S  L  R  G  I  I  P  L  E  N
661  GGTGTTAGAAAGACTTTGGACTACTGGCCATCCTTGAGAGGTATCATCCCATTGGAAAAC

     V  D  H  H  H  H  H  H  *  E  F  T  W  P  S  R
721  GTTGATCACCACCACCATCACCACTAAGAATTCACGTGGCCCAGCCGG
```

Figure A9: DNA fragment of rPA2 used for cloning into pPICZαA.

The amino acid sequence of rPA2 is underlined. rPA2 contained two additional N-terminal amino acids (EF) due to cloning into EcoRI restriction site. Linker (VD) and His_6-tag are written in italics. N56D is shown in red.

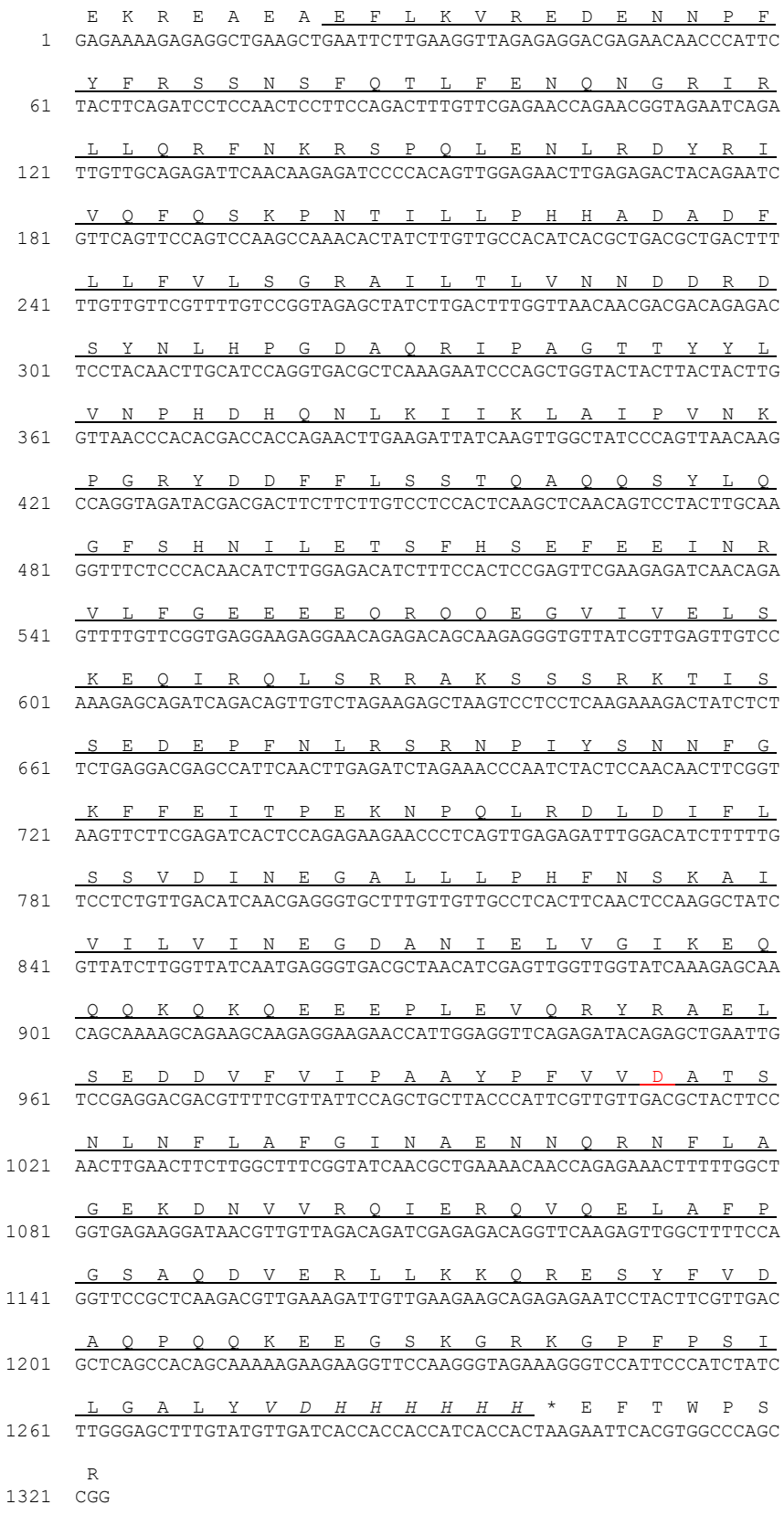


Figure A10: DNA fragment of rGly m 5.03 used for cloning into pPICZαA.

The amino acid sequence of rGly m 5.03 is underlined. rGly m 5.03 contained two additional N-terminal amino acids (EF) due to cloning into EcoRI restriction site. Linker (VD) and His_6-tag are written in italics. N328D is shown in red.

```
     E  K  R  E  A  E  A  E  F  S  K  W  Q  H  Q  Q  D  S  C  R
1    GAGAAAAGAGAGGCTGAAGCTGAATTCTCCAAGTGGCAACACCAACAGGACTCTTGTAGA

     K  Q  L  Q  G  V  N  L  T  P  C  E  K  H  I  M  E  K  I  Q
61   AAGCAGTTGCAGGGTGTTAACTTGACTCCATGTGAGAAGCACATCATGGAAAAGATCCAG

     G  R  G  D  D  D  D  D  D  D  D  D  N  H  I  L  R  T  M  R
121  GGTAGAGGTGATGACGACGATGATGACGATGACGACAACCACATCTTGAGAACTATGAGA

     G  R  I  N  Y  I  R  R  N  E  G  K  D  E  D  E  E  E  E  G
181  GGTAGAATCAACTACATCAGAAGAAACGAGGGTAAGGACGAGGACGAAGAGGAAGAGGGT

     H  M  Q  K  C  C  T  E  M  S  E  L  R  S  P  K  C  Q  C  K
241  CATATGCAAAAGTGTTGTACTGAGATGTCCGAGTTGAGATCCCCAAAGTGTCAGTGTAAG

     A  L  Q  K  I  M  E  D  Q  S  E  E  L  E  E  K  Q  K  K  K
301  GCTTTGCAAAAGATCATGGAAGATCAGTCCGAAGAGTTGGAAGAGAAGCAGAAAAAGAAG

     M  E  K  E  L  I  N  L  A  T  M  C  R  F  G  P  M  I  Q  C
361  ATGGAAAAAGAGTTGATTAACTTGGCTACTATGTGTAGATTCGGTCCAATGATCCAGTGT

     D  L  S  S  D  D  V  D  H  H  H  H  H  H  *  E  F  T  W  P
421  GACTTGTCCTCTGATGATGTTGATCACCACCACCATCACCACTAAGAATTCACGTGGCCC

     S  R
481  AGCCGG
```

Figure A11: DNA fragment of rGly m 8 used for cloning into pPICZαA.

The amino acid sequence of rGly m 8 is underlined. rGly m 8 contained two additional N-terminal amino acids (EF) due to cloning into EcoRI restriction site. Linker (VD) and His_6-tag are written in italics. Propeptide is shown in blue and N99D is shown in red.

7.1.3 SDS-PAGE of rPis s 1 expressed in *Pichia pastoris*

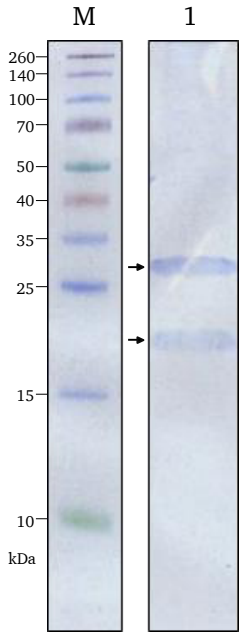


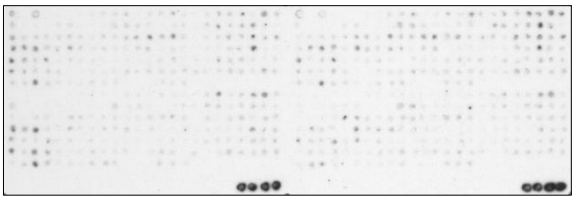
Figure A12: SDS-PAGE of rPis s 1 expressed in *Pichia pastoris*.

Coomassie-stained SDS-PAGE of rPis s 1 secreted into cell culture supernatant. rPis s 1 (lane 1; 12 μl cell culture supernatant) was analyzed under reducing conditions. Arrows indicate the two truncated proteolytic fragments of rPis s 1. M, Spectra™ Multicolor Broad Range Protein Ladder.

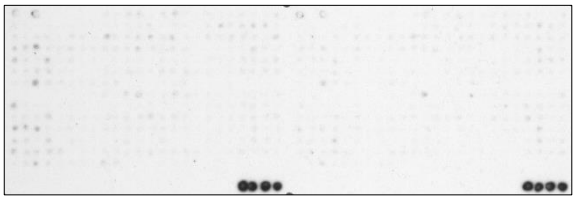
7.1.4 IgE immunodetection of multipeptide microarrays

7.1.4.1 Ara h 1 multipeptide microarray

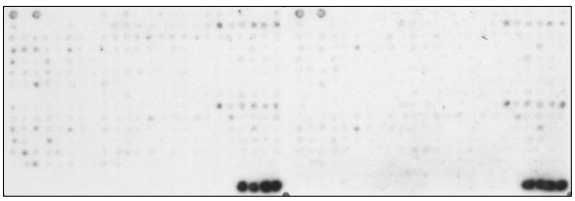
A



Patient 1 (2 min)



Patient 2 (2 min)



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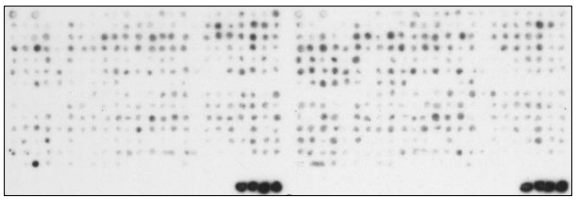

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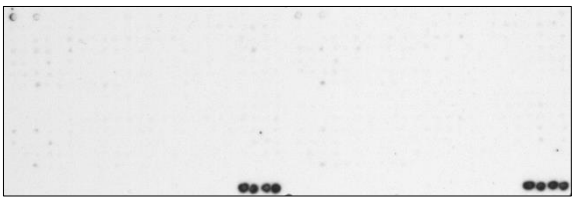

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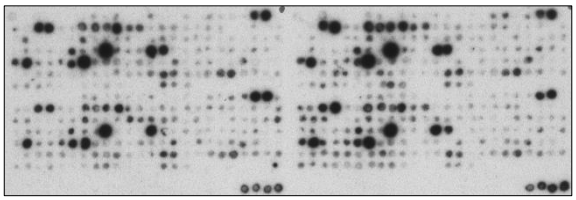
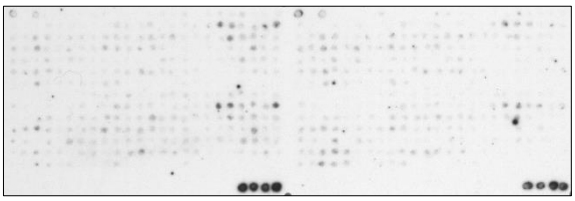


Patient 6 (30 sec)



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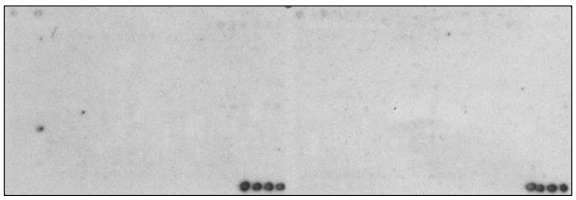
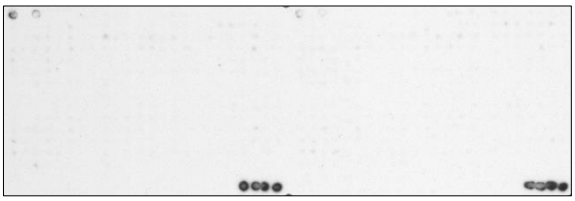


Patient 8 (30 sec)



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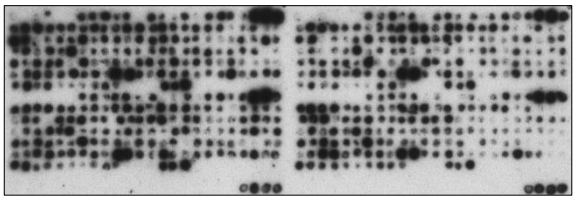

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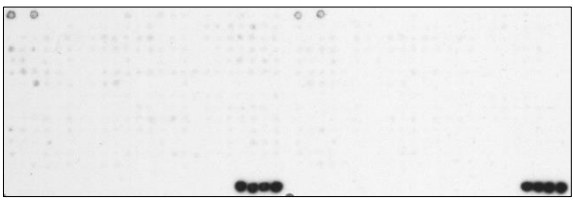

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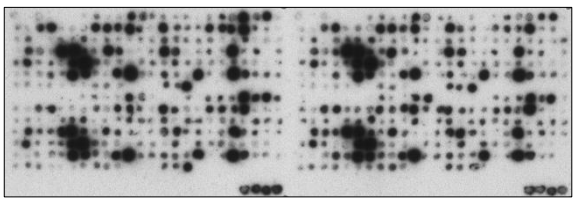
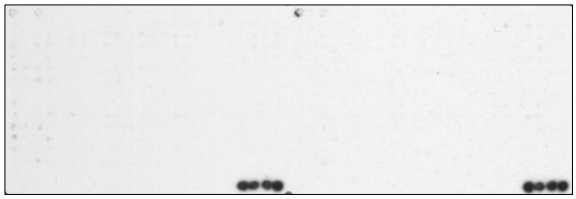
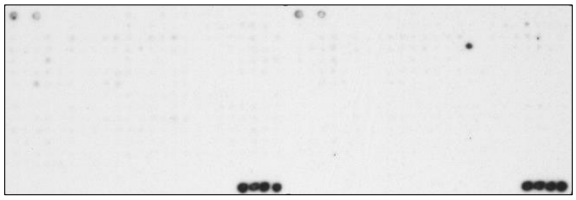


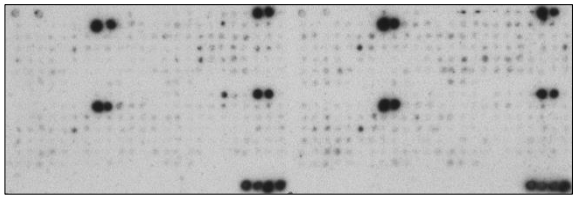
Patient 12 (30 sec)



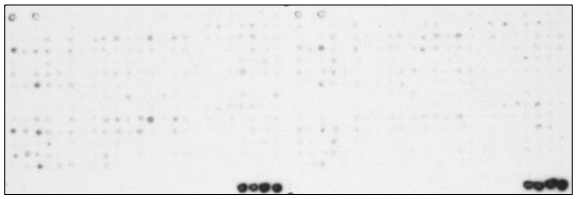
Patient 13 (2 min)



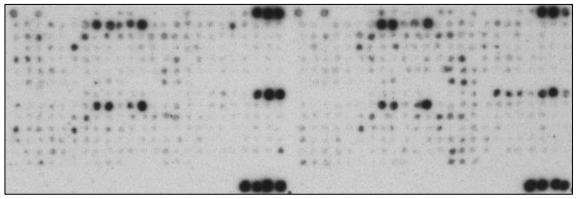
Patient 14 (2 min)



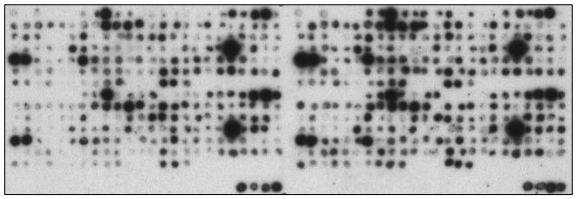
Patient 15 (2 min)



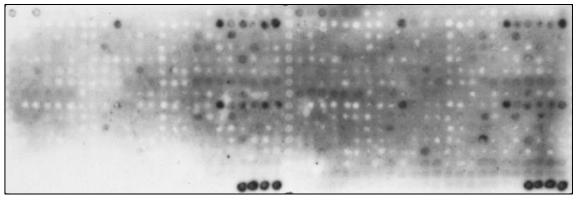
Patient 16 (2 min)



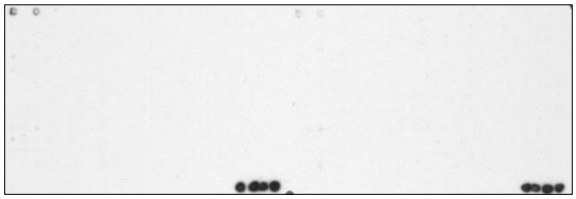
Patient 17 (2 min)



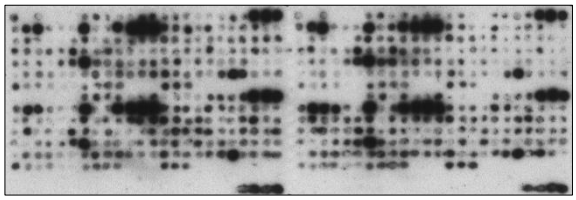
Patient 18 (30 sec)



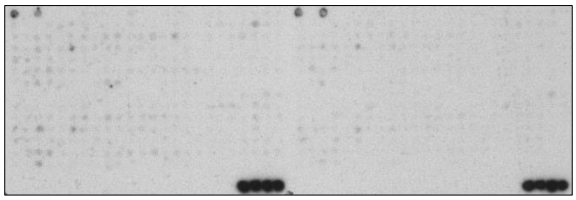
Patient 19 (30 sec)



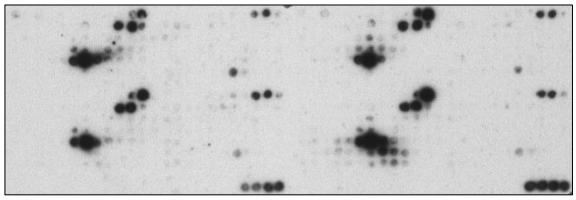
Patient 20 (2 min)



Patient 21 (30 sec)

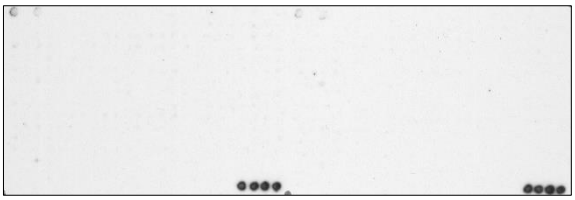


Patient 22 (2 min)

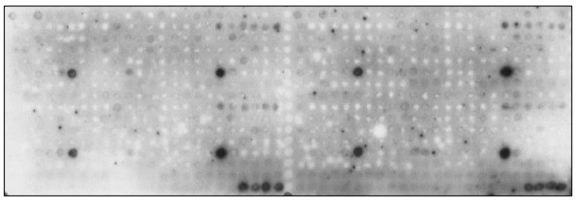


Patient 23 (30 sec)

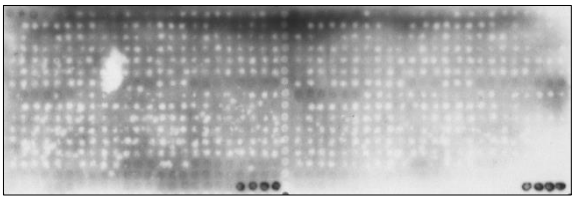
B



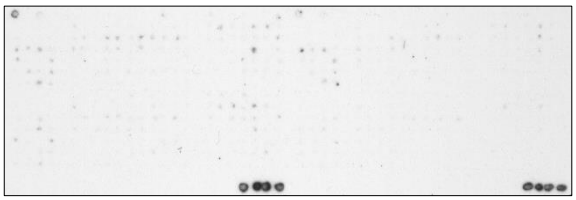
Patient 24 (2 min)



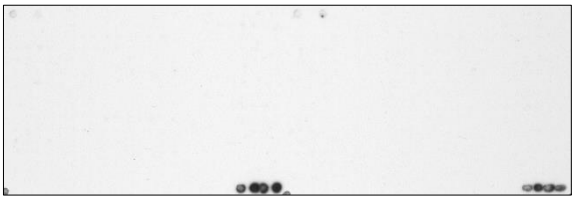
Patient 25 (30 sec)



Patient 26 (30 sec)

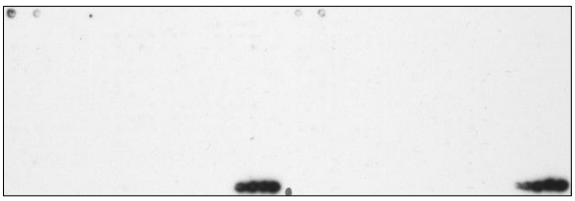


Patient 27 (2 min)

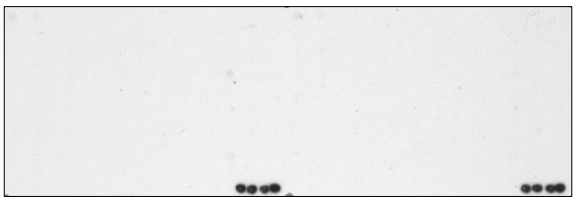


Patient 28 (2 min)

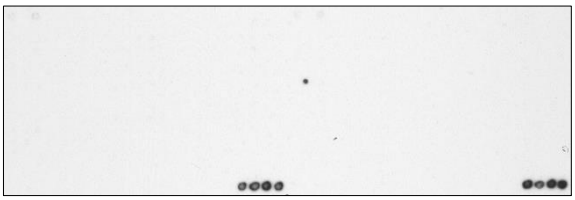
Patient 29 (2 min)



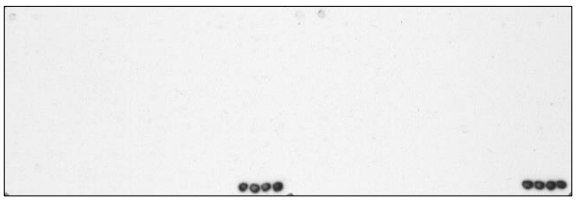
Patient 30 (2 min)



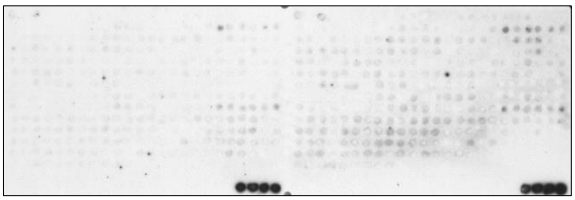
Patient 31 (2 min)



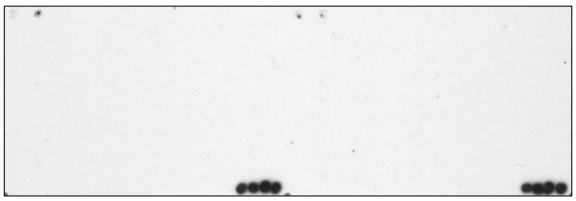
Patient 32 (2 min)



Patient 33 (2 min)



Patient 34 (2 min)



Patient 35 (2 min)

C

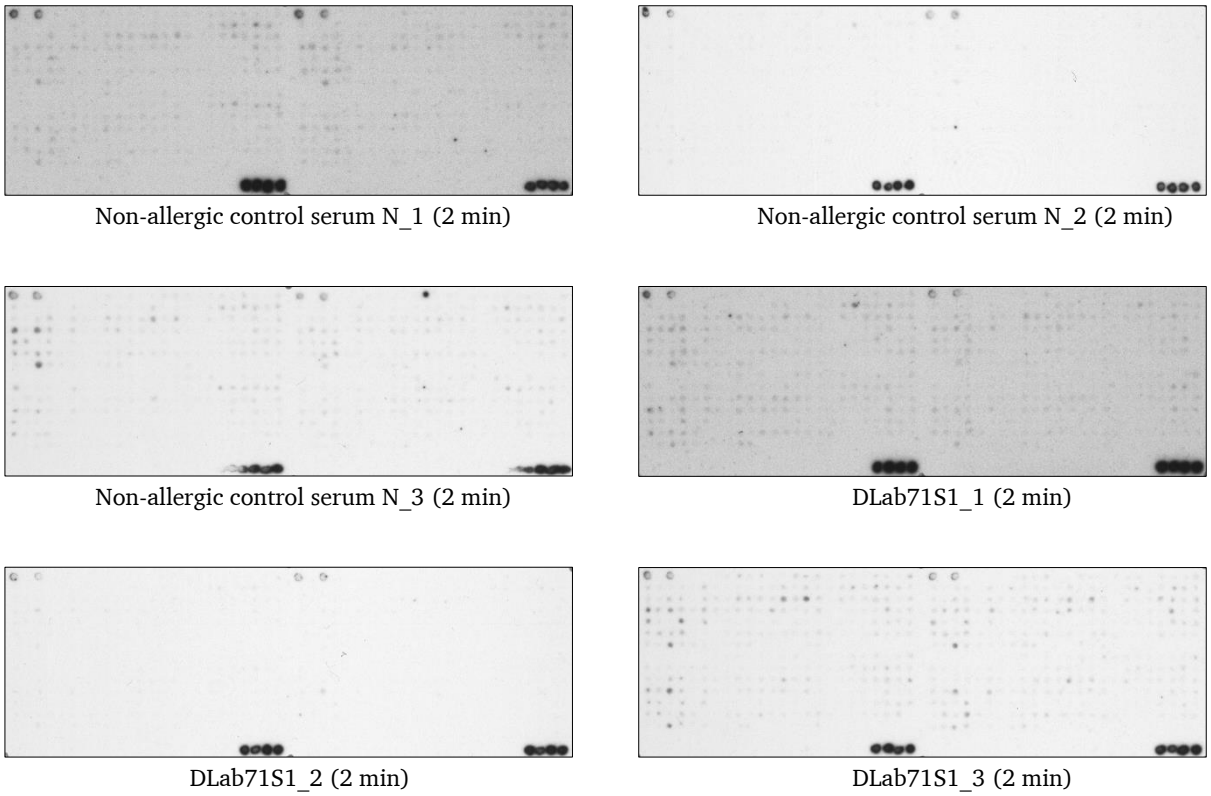
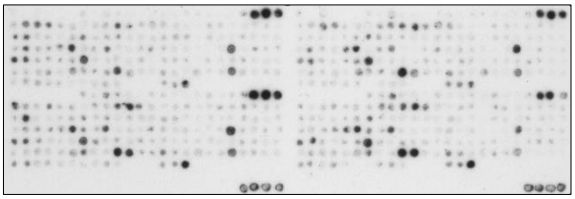
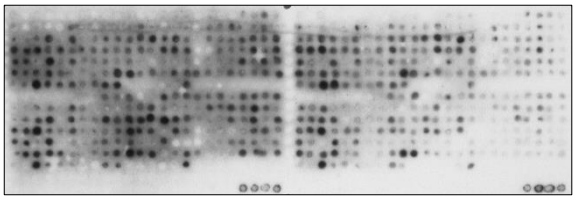


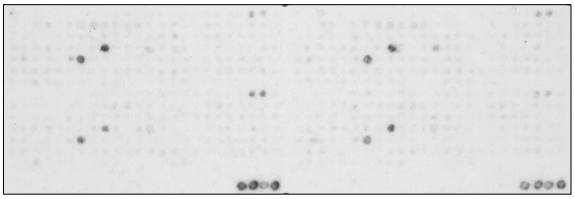
Figure A13: IgE immunodetection of Ara h 1 peptides using peanut-allergic, peanut-tolerant and control sera. Peptides representing Ara h 1 were spotted in quadruplicate and analyzed for their IgE binding using sera from peanut-allergic children (A), peanut-sensitized but tolerant children (B) and control sera (C). The exposure times are shown in parentheses and ranged from 30 sec to 2 min. N_1, N_2 and N_3 mean serum from non-allergic control N used on different X-ray films. The same applies for DLab71S1_1, DLab71S1_2 and DLab71S1_3.



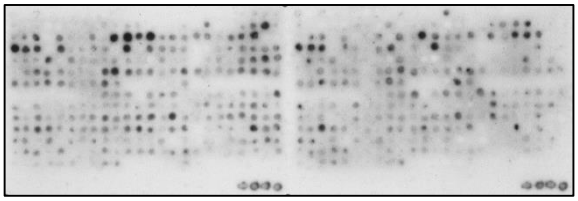
Serum pool 1 + protein buffer (30 sec)



Serum pool 1 + rAra h 1 (30 sec)



Serum pool 2 + protein buffer (30 sec)



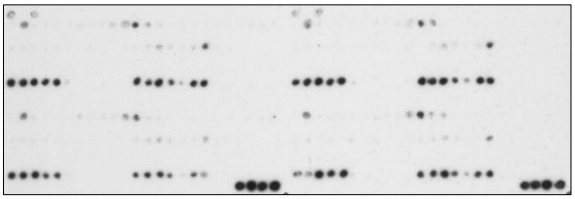
Serum pool 2 + rAra h 1 (30 sec)

Figure A14: Inhibition of IgE binding to Ara h 1 peptides.

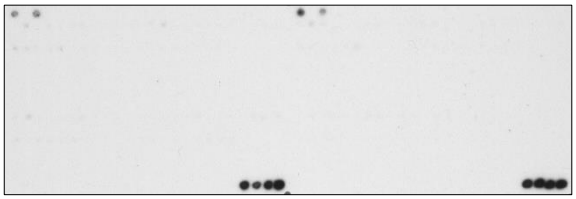
Verification of the specificity of IgE binding to Ara h 1 peptides using two serum pools preincubated with 20.5 μg rAra h 1. Serum pool 1 was composed of sera from patients 10, 12, 18, 21 and serum pool 2 of sera from patients 6, 15, 17, 23. As a reference, the respective uninhibited serum pool plus protein buffer without rAra h 1 was used. The exposure times are shown in parentheses.

7.1.4.2 Ara h 2 multipeptide microarray

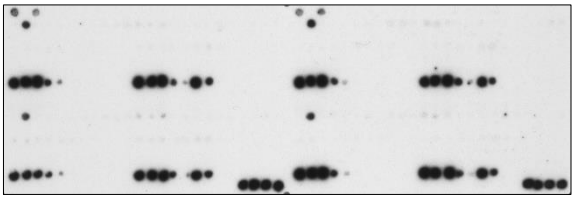
A



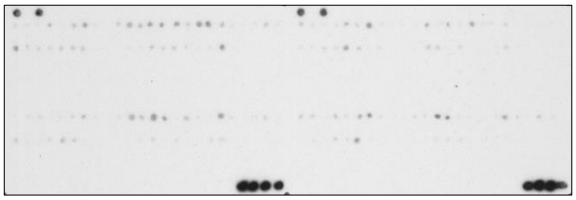
Patient 1 (2 min)



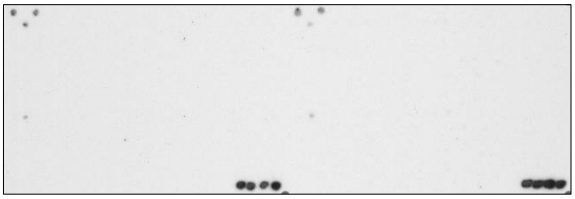
Patient 2 (2 min)



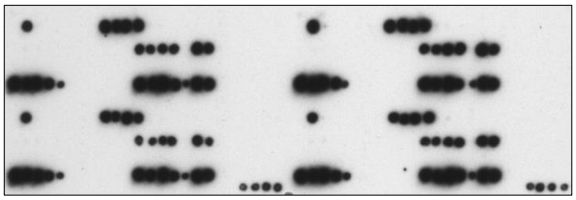
Patient 3 (2 min)



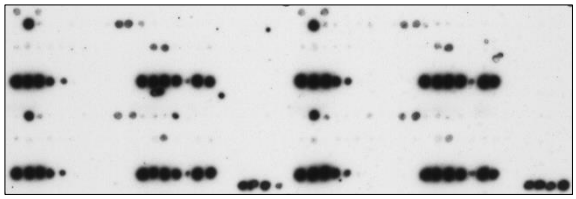
Patient 4 (2 min)



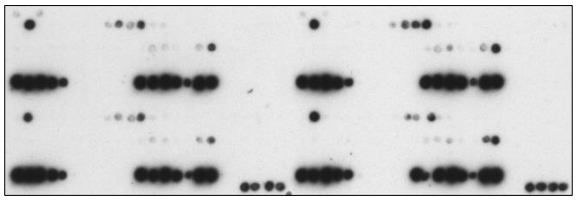
Patient 5 (2 min)



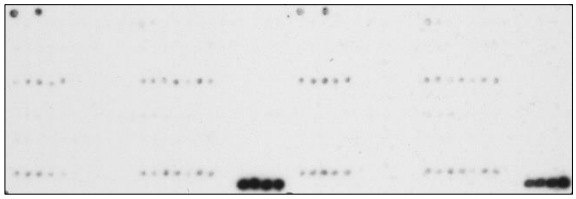
Patient 6 (30 sec)



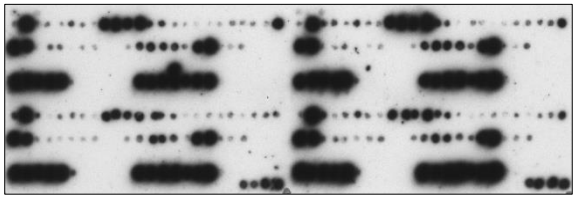
Patient 7 (2 min)



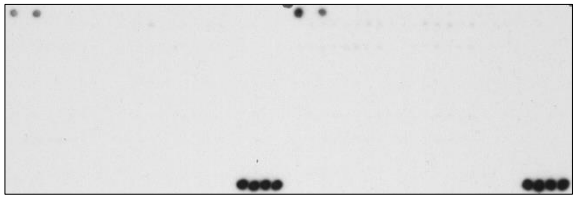
Patient 8 (30 sec)



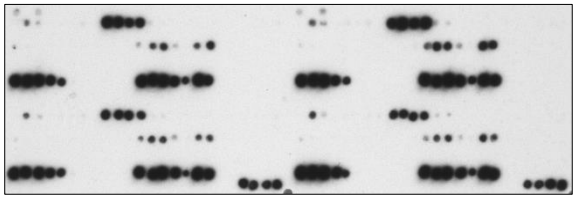
Patient 9 (2 min)



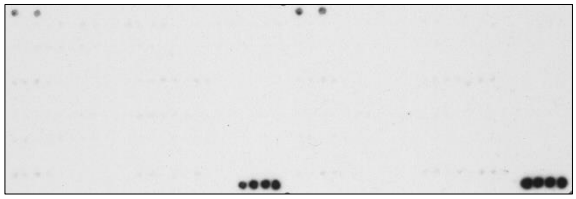
Patient 10 (30 sec)



Patient 11 (2 min)



Patient 12 (30 sec)



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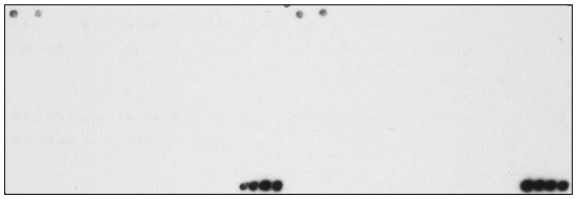
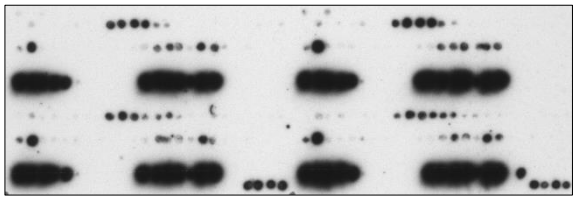


Patient 14 (2 min)



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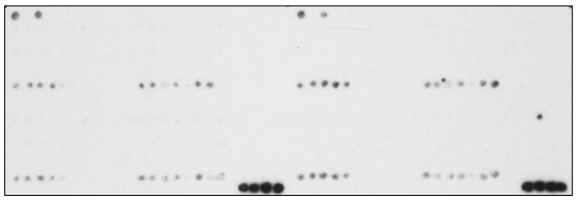
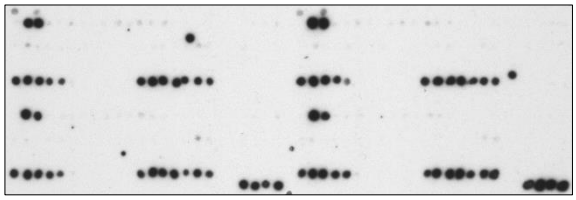


Patient 16 (2 min)



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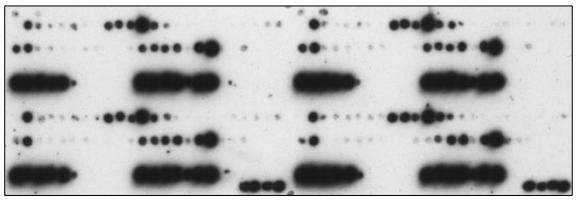

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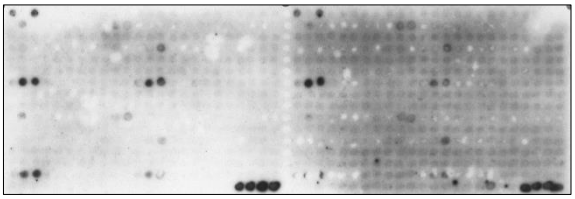

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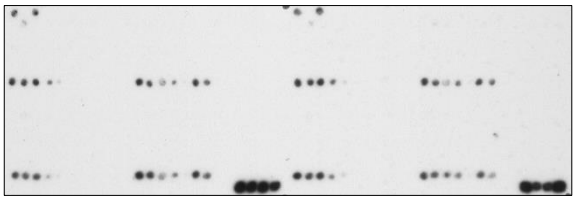
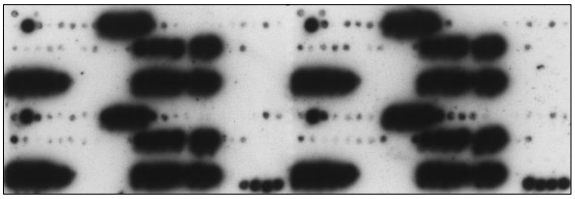
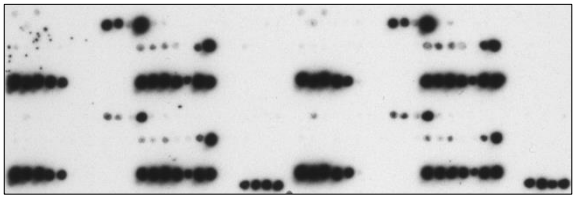


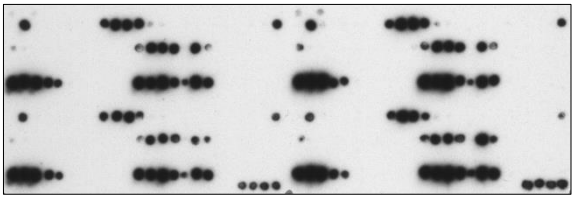
Patient 20 (2 min)



Patient 21 (30 sec)



Patient 22 (30 sec)



Patient 23 (30 sec)

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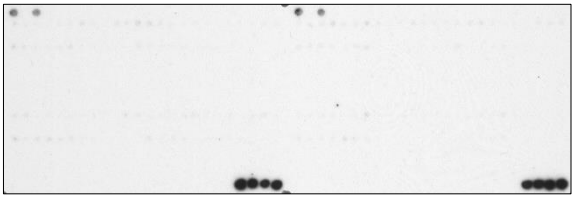
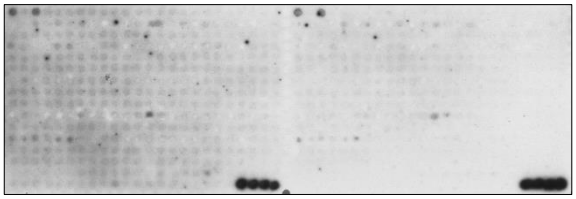
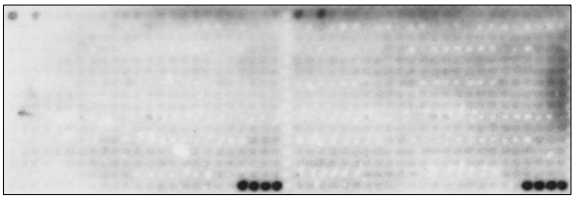


Patient 24 (2 min)



Patient 25 (1 min)



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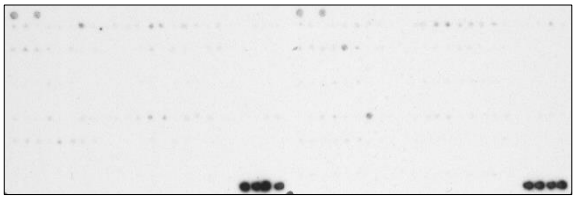
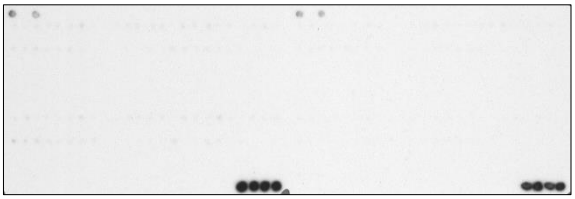
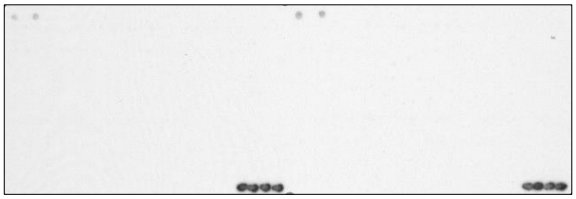


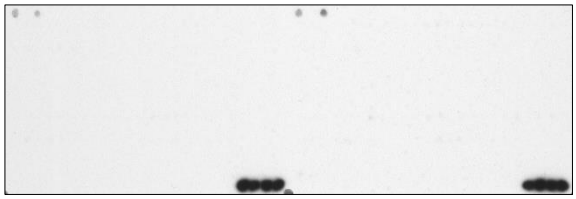
Patient 27 (2 min)



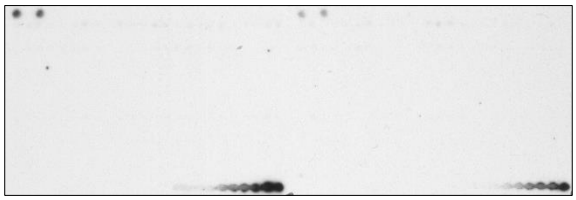
Patient 28 (2 min)



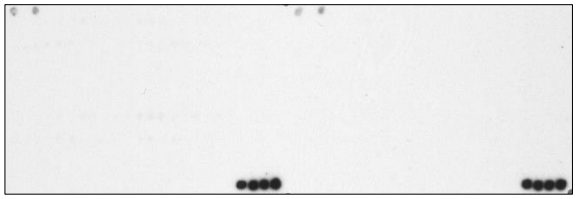
Patient 29 (2 min)



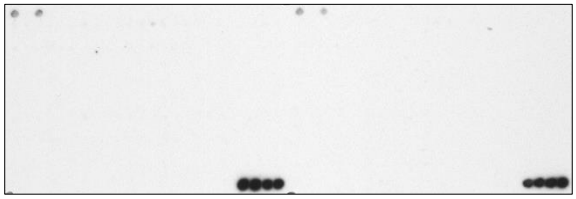
Patient 30 (2 min)



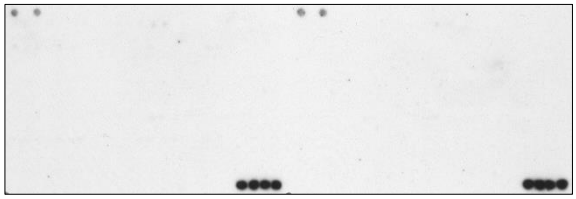
Patient 31 (2 min)



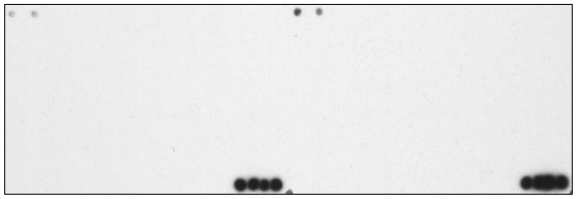
Patient 32 (2 min)



Patient 33 (2 min)

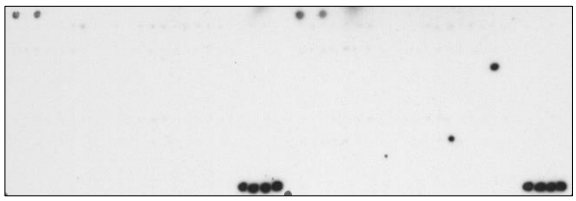


Patient 34 (2 min)

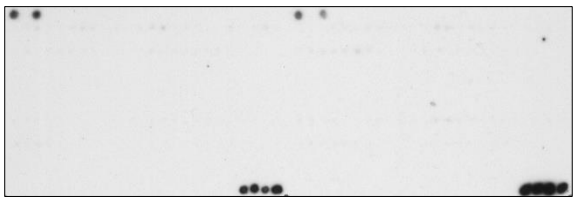


Patient 35 (2 min)

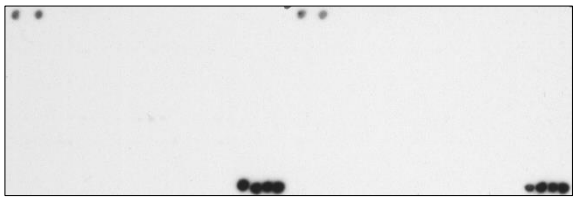
C



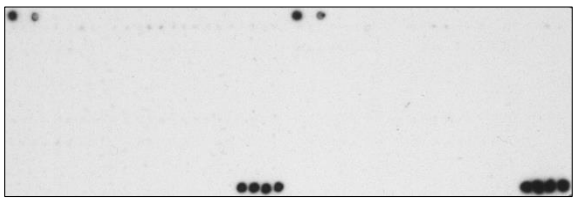
Non-allergic control serum N_2 (2 min)



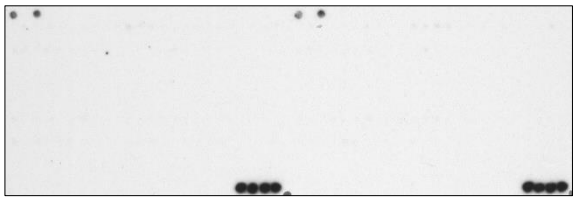
Non-allergic control serum N_3 (2 min)



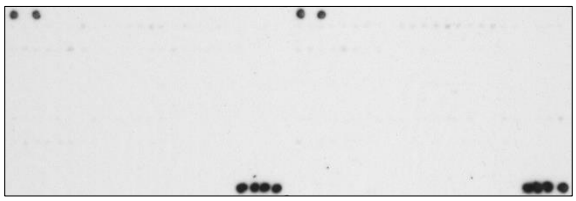
Non-allergic control serum N_4 (2 min)



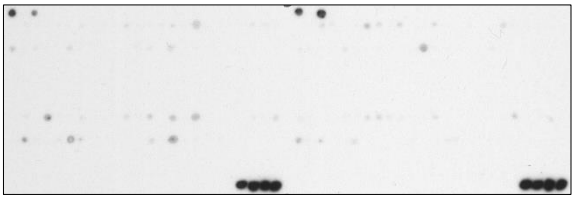
DLab71S1_1 (2 min)



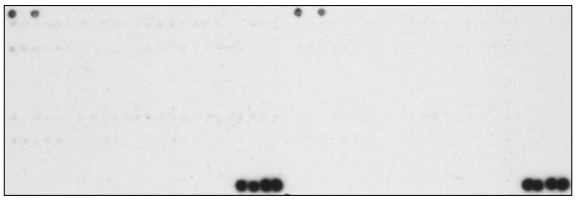
DLab71S1_2 (2 min)



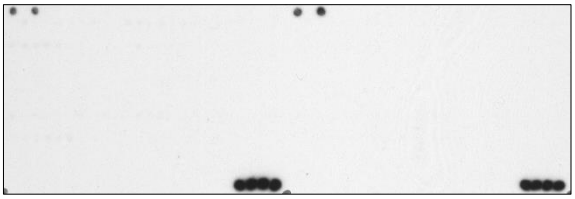
DLab71S1_3 (2 min)



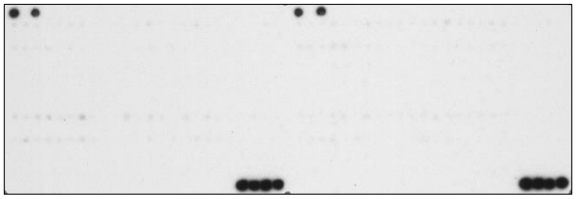
DLab71S1_4 (2 min)



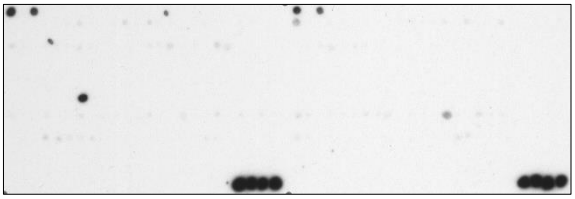
DLab72S1_1 (2 min)



DLab72S1_2 (2 min)

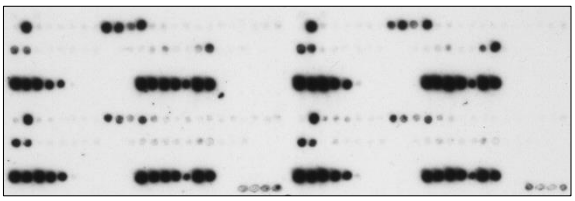


DLab72S1_3 (2 min)

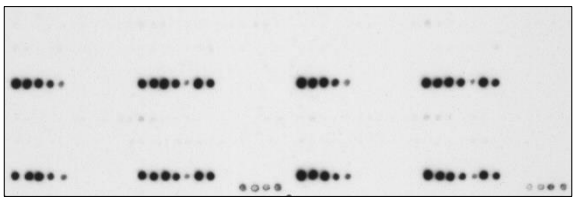


DLab72S1_4 (2 min)

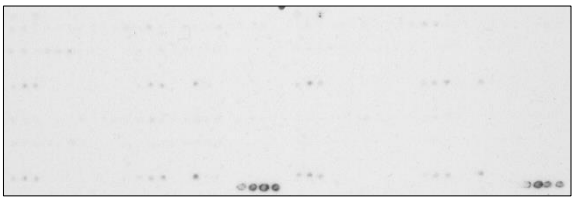
Figure A15: IgE immunodetection of Ara h 2 peptides using peanut-allergic, peanut-tolerant and control sera.
Peptides representing Ara h 2.01_P, Ara h 2.01_Hyp, Ara h 2.02_P and Ara h 2.02_Hyp were spotted in quadruplicate and analyzed for their IgE binding using sera from peanut-allergic children (A), peanut-sensitized but tolerant children (B) and control sera (C). The exposure times are shown in parentheses and ranged from 30 sec to 2 min. Numbers written behind control sera represent the respective developed X-ray film.



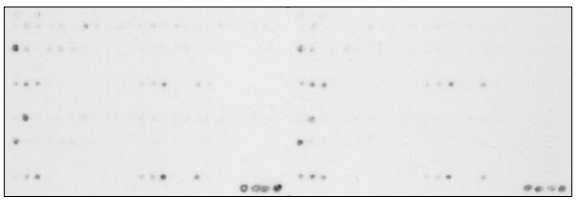
Serum pool + protein buffer (30 sec)



Serum pool + rAra h 2.02 (30 sec)



Serum pool + native peanut extract (30 sec)

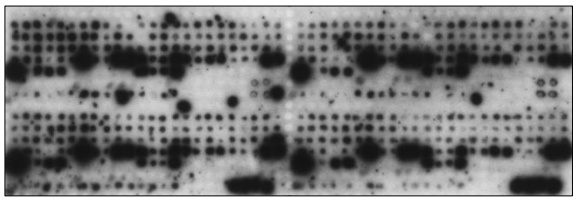


Serum pool + red./alk. peanut extract (30 sec)

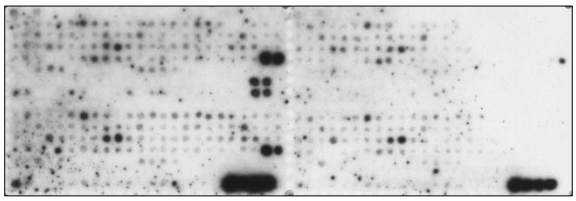
Figure A16: Inhibition of IgE binding to Ara h 2 peptides.
Verification of the specificity of IgE binding to Ara h 2 peptides using one serum pool preincubated with 13.5 μg rAra h 2.02, 9.5 μg native peanut extract or 9.5 μg reduced/alkylated peanut extract. The used serum pool was composed of sera from patients 6, 7, 8, 10, 12, 15, 18, 21, 22 and 23. As a reference, uninhibited serum pool plus protein buffer without inhibitor was used. The exposure times are shown in parentheses.

7.1.4.3 Pis s 1 multipeptide microarray

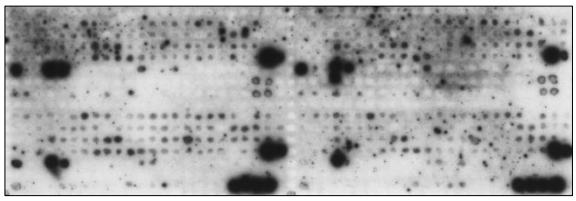
A



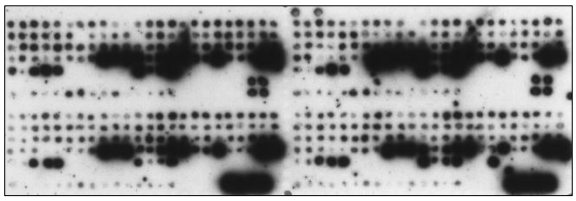
Patient 1 (30 sec)



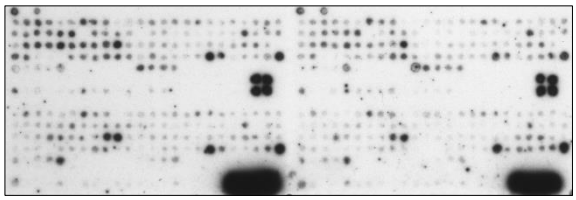
Patient 2 (2 min)



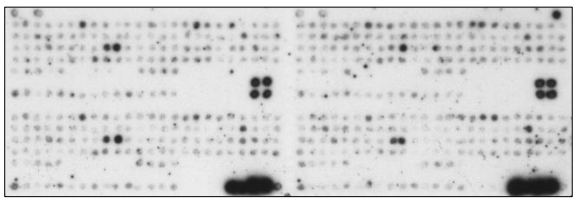
Patient 3 (1 min)



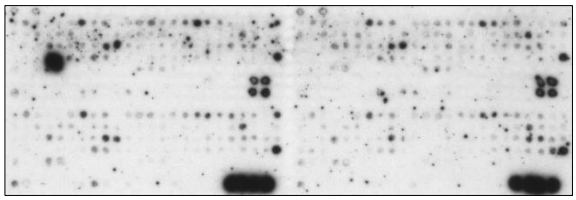
Patient 4 (30 sec)



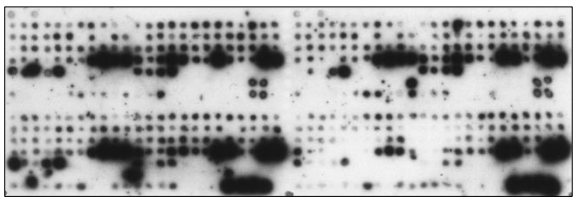
Patient 5 (2 min)



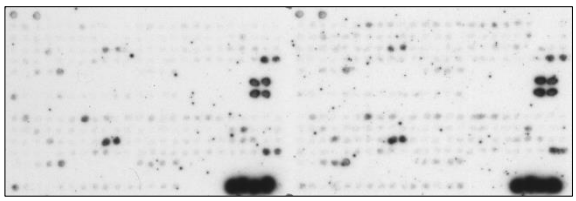
Patient 6 (2 min)



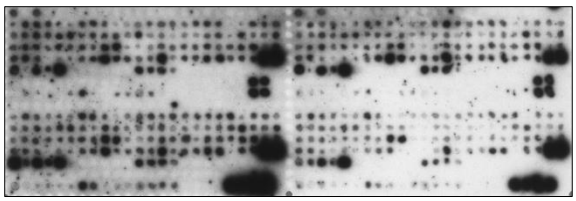
Patient 7 (2 min)



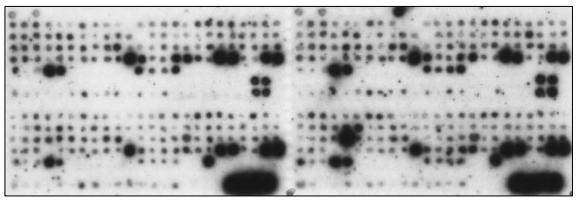
Patient 8 (30 sec)



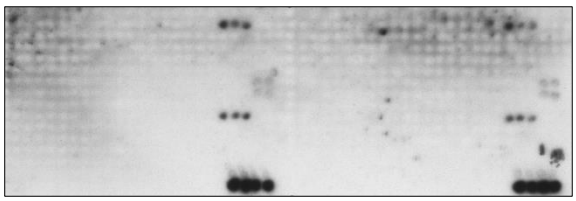
Patient 9 (2 min)



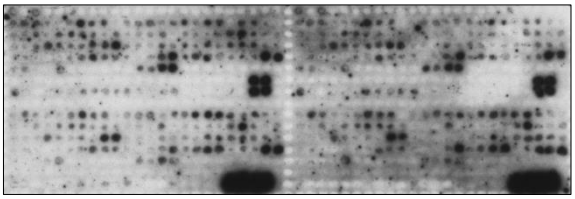
Patient 10 (2 min)



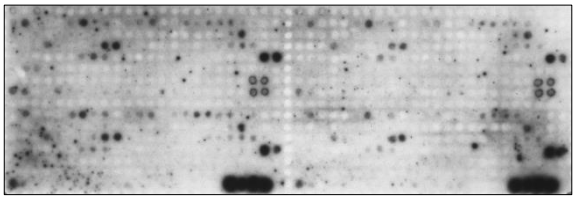
Patient 11 (2 min)



Patient 12 (2 sec)

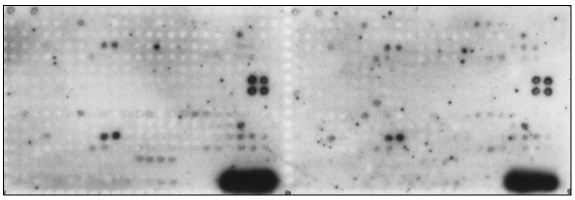


Patient 13 (2 min)

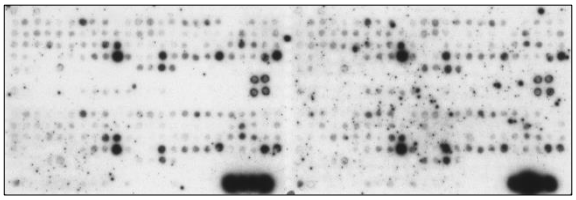


Patient 14 (2 min)

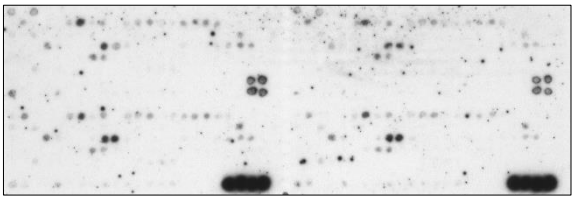
B



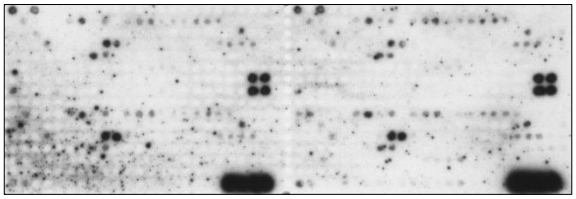
Patient 15 (2 min)



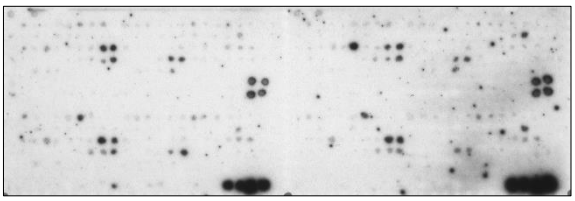
Patient 16 (2 min)



Patient 17 (2 min)

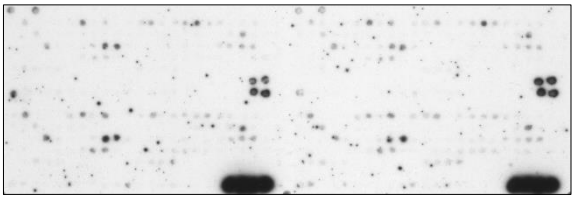


Patient 18 (2 min)

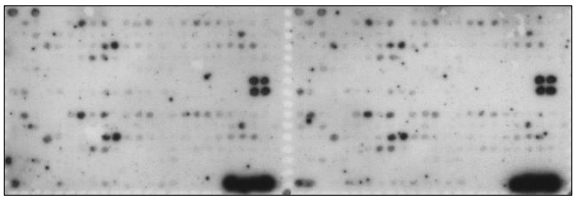


Patient 19 (2 min)

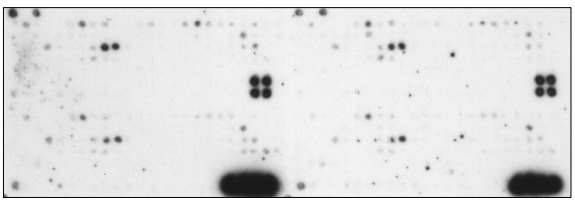
C



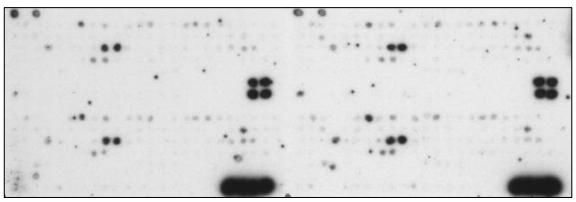
Serum A (2 min)



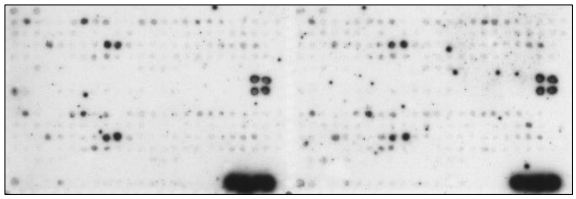
Serum B (2 min)



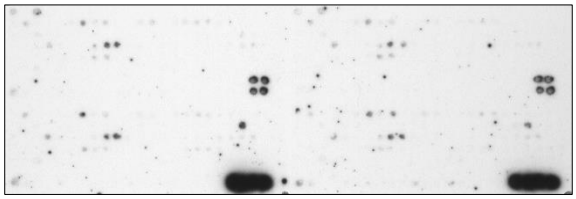
Serum C (2 min)



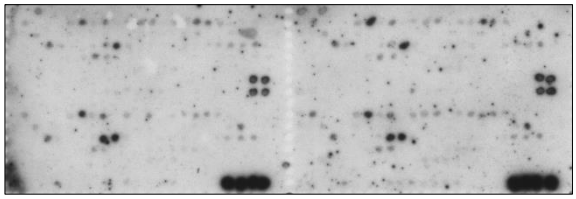
Serum D (2 min)



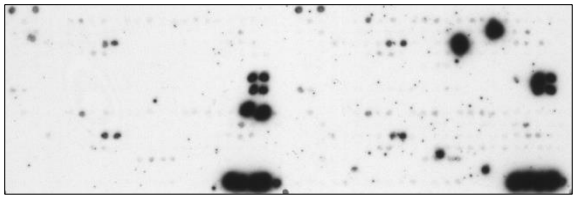
Serum E (2 min)



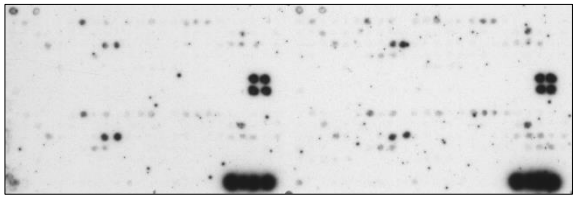
Serum F (2 min)



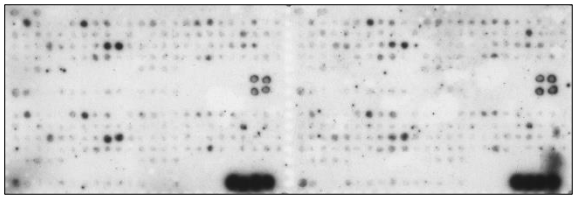
Serum G (2 min)



Serum H (2 min)

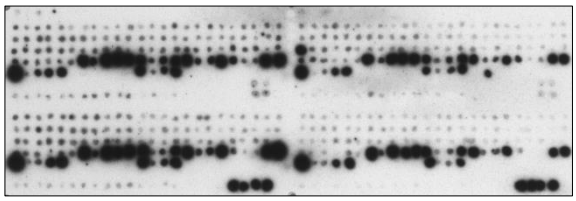


Serum I (2 min)

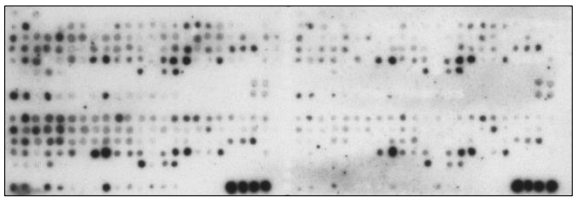


Serum J (2 min)

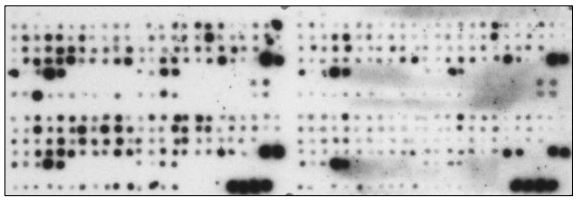
Figure A17: IgE immunodetection of Pis s 1 peptides using pea-allergic, pea-tolerant and control sera.
Peptides representing Pis s 1 were spotted in quadruplicate and analyzed for their IgE binding using sera from pea-allergic children (A), pea-sensitized but tolerant children (B) and control sera (C). The exposure times are shown in parentheses and ranged from 2 sec to 2 min.



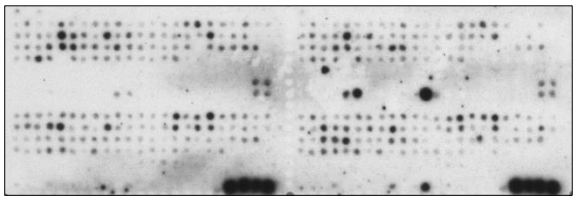
Serum pool 1 + protein buffer (1 min)



Serum pool 1 + rPis s 1 (1 min)



Serum pool 2 + protein buffer (1 min)

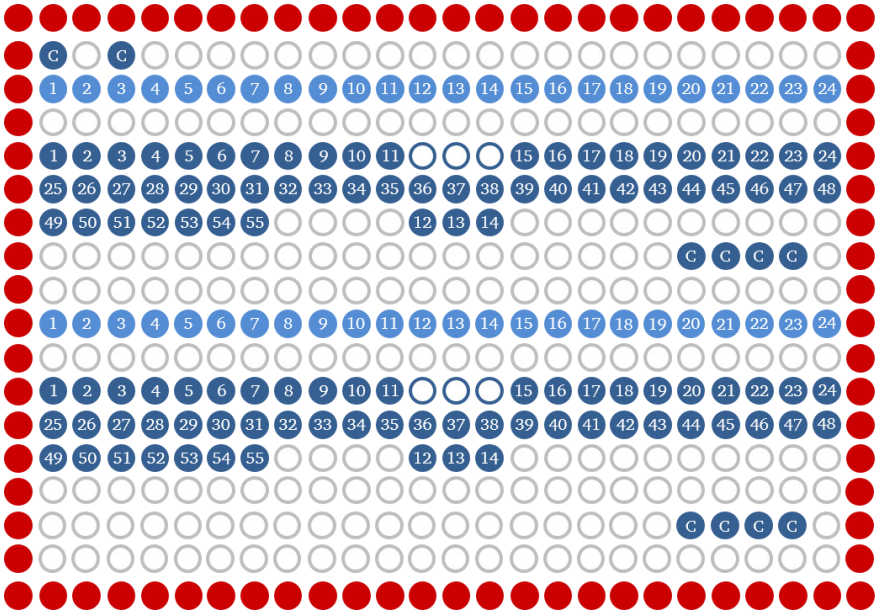


Serum pool 2 + rPis s 1 (1 min)

Figure A18: Inhibition of IgE binding to Pis s 1 peptides.
Verification of the specificity of IgE binding to Pis s 1 peptides using two serum pools preincubated with 30 μg rPis s 1. Serum pool 1 was composed of sera from patients 1, 4, 8 and serum pool 2 of sera from patients 3, 10, 11, 13. As a reference, the respective uninhibited serum pool plus protein buffer without inhibitor was used. The exposure times are shown in parentheses.

7.1.4.4 PA1/PA2 multipeptide microarray

A



B

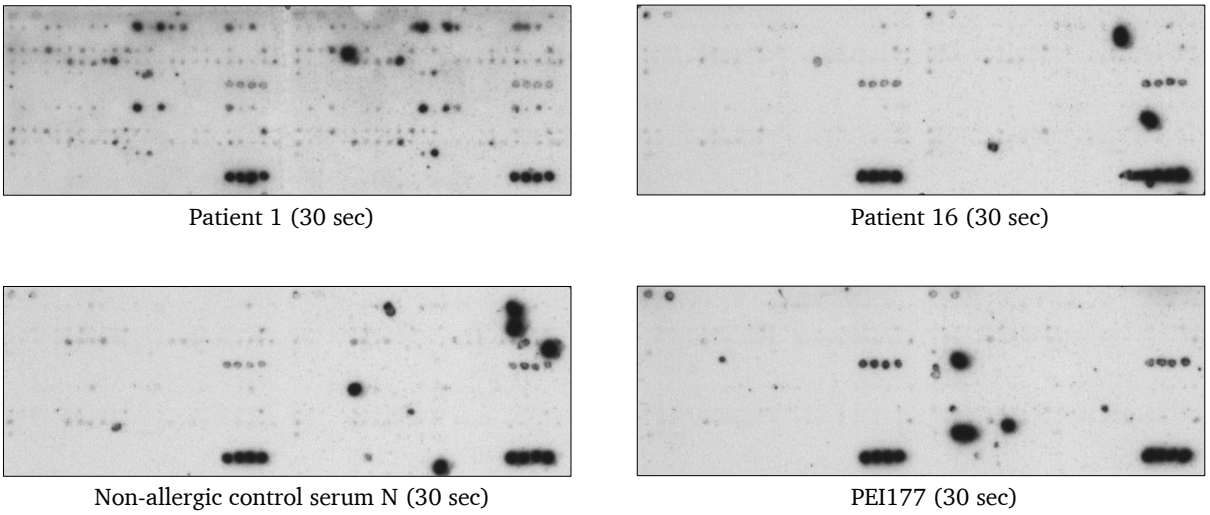
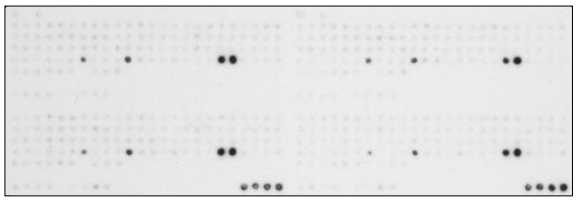


Figure A19: Spotting layout and IgE immunodetection of PA1 and PA2 peptides using pea-allergic, pea-tolerant and control sera.

(A) Spotting layout of PA1 and PA2 multipeptide microarray. For simplification, only the left segment of the multipeptide microarray is shown. Peptide spots representing PA1 and PA2 are shown in light blue and dark blue, respectively. PA1 and PA2 are represented by 24 and 55 peptides, respectively. Biotinylated control peptides, abbreviated by a "C", were spotted in duplicate in different dilutions (top left, middle right and bottom right on each array element) and were used as position markers. Gray empty spots represent blank spots composed of peptide printing buffer (DMSO). Blue empty spots represent internal control peptides not relevant for peptide analysis. (B) IgE immunodetection of patient 1 (allergic), patient 16 (tolerant) and two control sera (serum N and PEI177) after 30 sec exposure.

7.1.4.5 Gly m 5.03 multipeptide microarray

A



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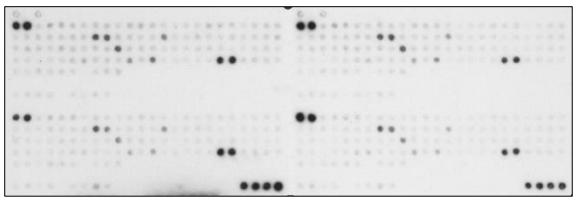

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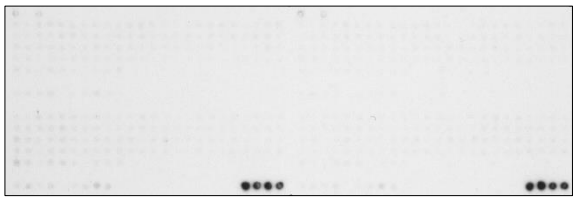

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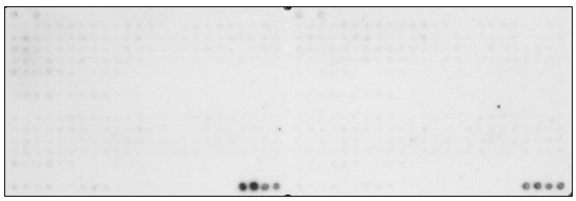

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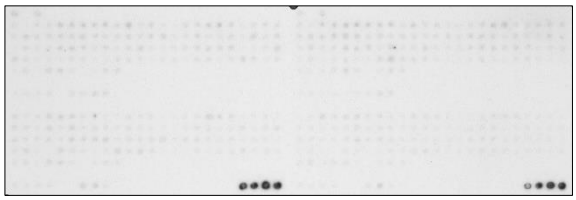
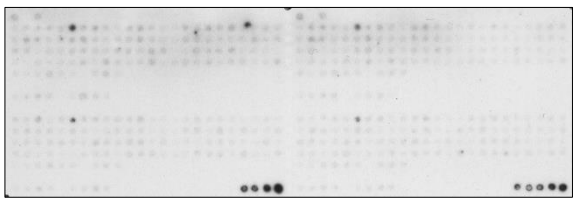


Patient 5 (30 sec)

Patient 6 (30 sec)

B



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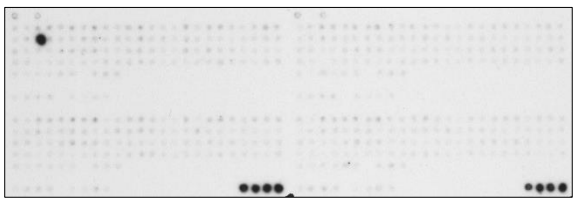

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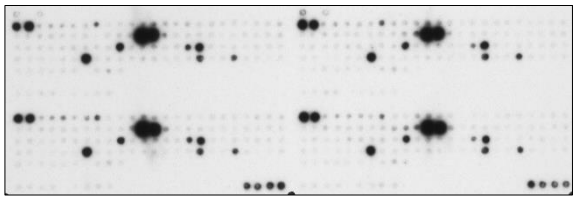

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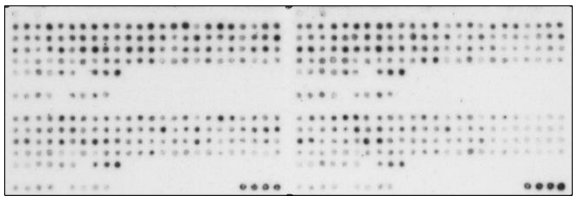
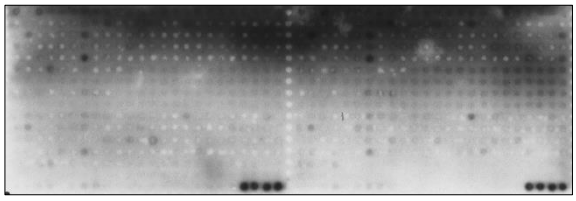


Patient 10 (30 sec)



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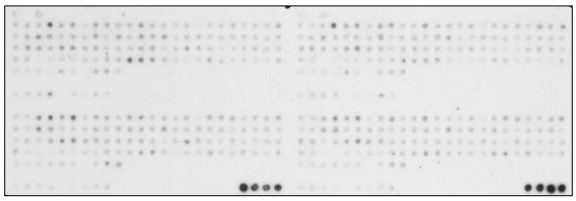

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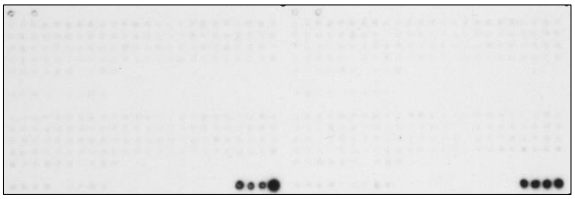

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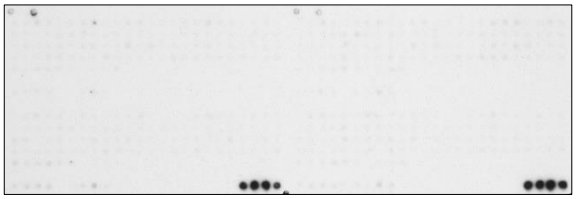
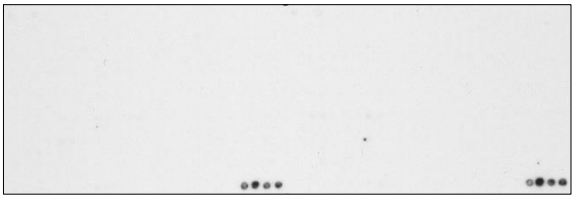
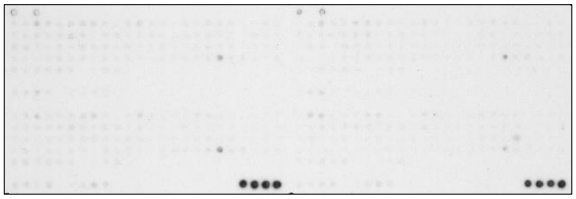


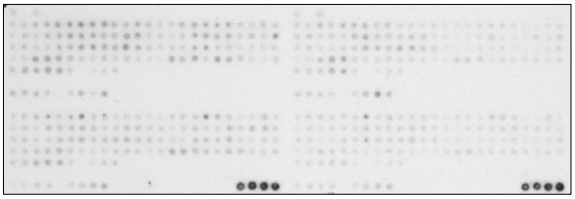
Patient 14 (30 sec)



Patient 15 (30 sec)

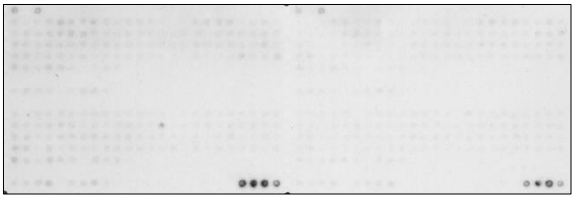


Patient 16 (30 sec)

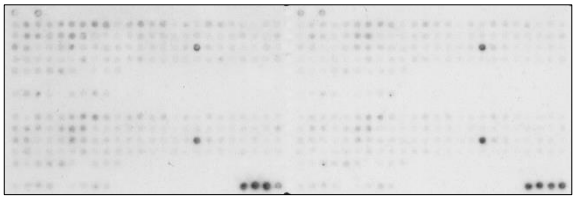


Patient 17 (30 sec)

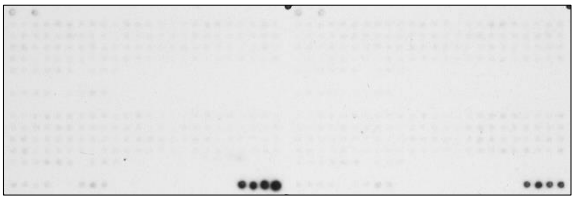
Patient 18 (30 sec)



Patient 19 (30 sec)

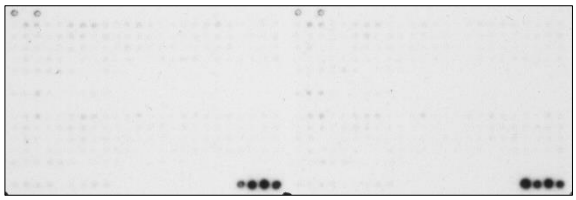


Patient 20 (30 sec)

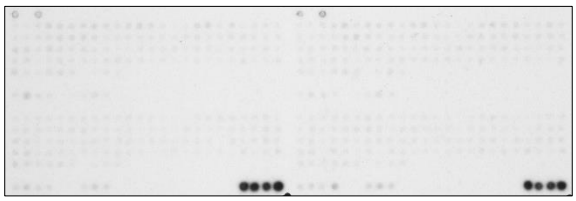


Patient 21 (30 sec)

C



Non-allergic control serum N_1 (30 sec)



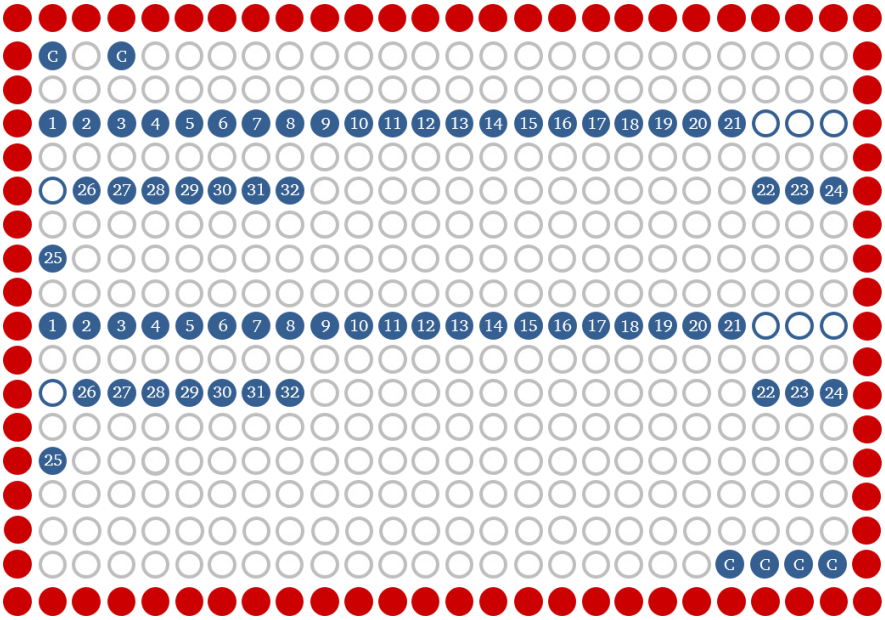
Non-allergic control serum N_2 (30 sec)

Figure A20: IgE immunodetection of Gly m 5.03 peptides using soybean-allergic, soybean-tolerant and control sera.

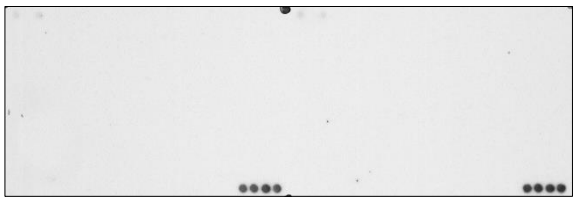
Peptides representing Gly m 5.0301 and Gly m 5.0302 were spotted in quadruplicate and analyzed for their IgE binding using sera from soybean-allergic children (A), soybean-sensitized but tolerant children (B) and control sera (C). The exposure times are shown in parentheses and ranged from 15 sec to 30 sec. Numbers written behind control serum represent the respective developed X-ray film.

7.1.4.6 Gly m 8 multipeptide microarray

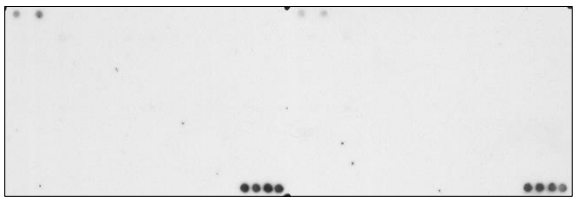
A



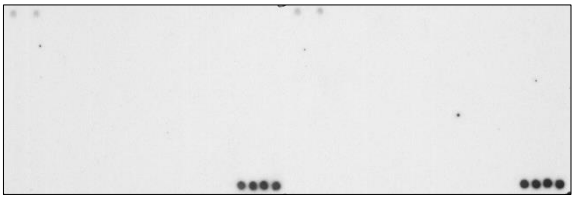
B



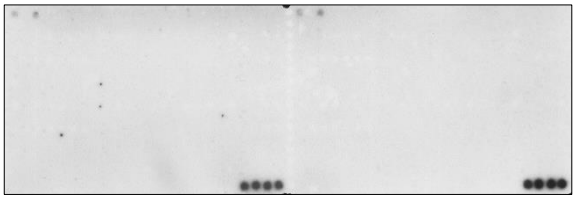
Patient 1 (30 sec)



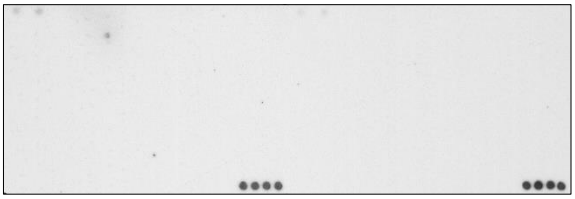
Patient 2 (30 sec)



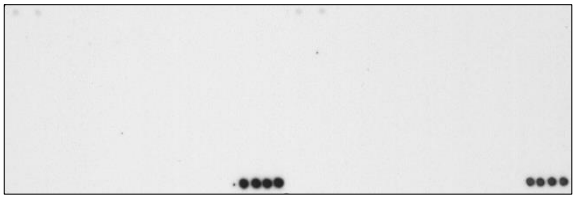
Patient 3 (30 sec)



Patient 4 (30 sec)

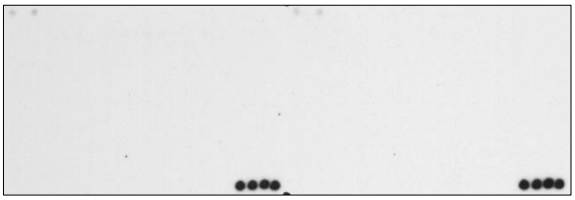


Patient 5 (30 sec)

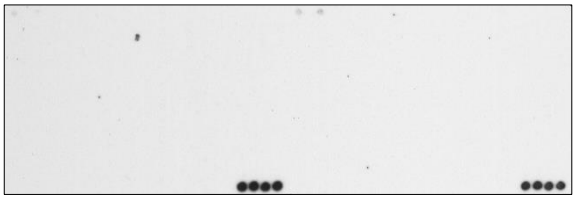


Patient 6 (30 sec)

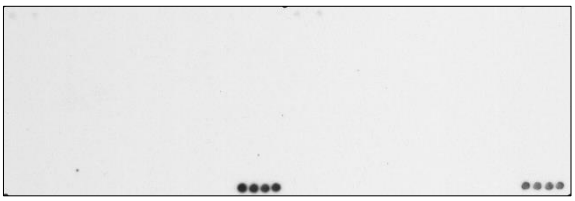
C



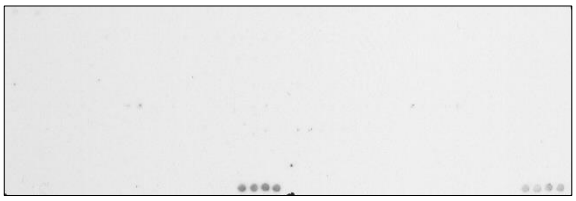
Patient 7 (30 sec)



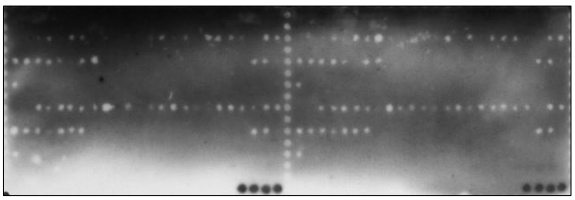
Patient 8 (30 sec)



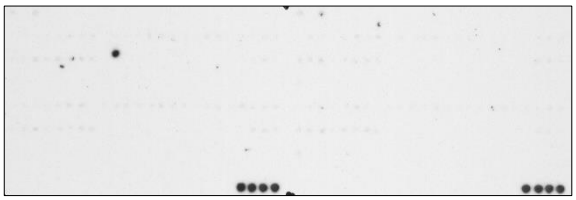
Patient 9 (30 sec)



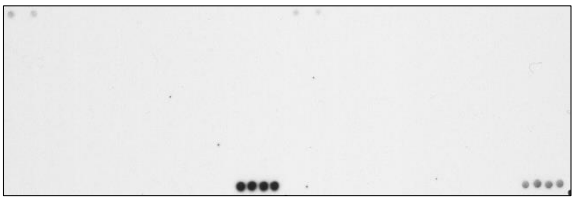
Patient 10 (30 sec)



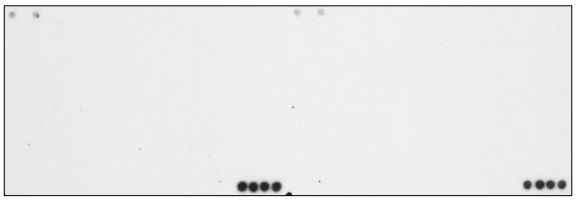
Patient 11 (15 sec)



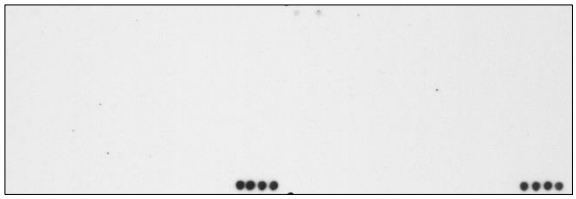
Patient 12 (30 sec)



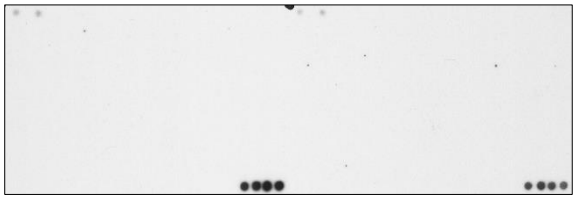
Patient 13 (30 sec)



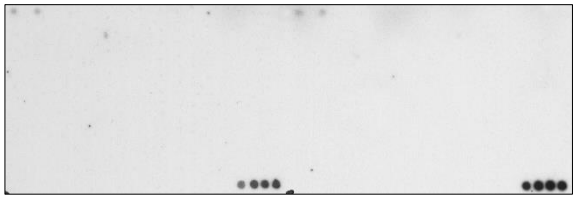
Patient 14 (30 sec)



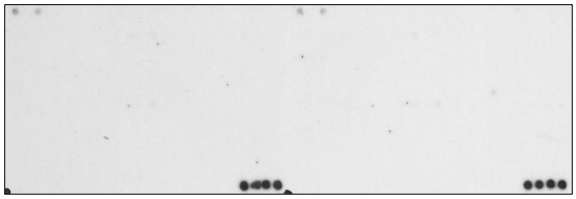
Patient 15 (30 sec)



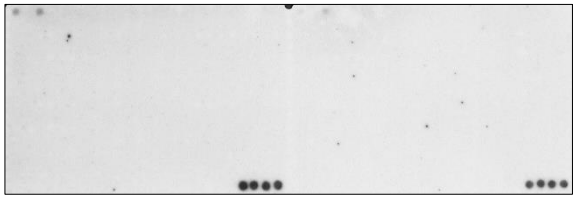
Patient 16 (30 sec)



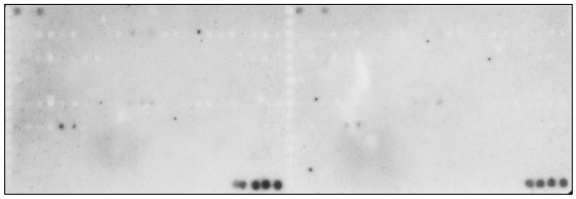
Patient 17 (30 sec)



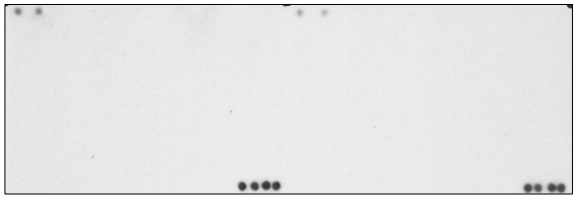
Patient 18 (30 sec)



Patient 19 (30 sec)

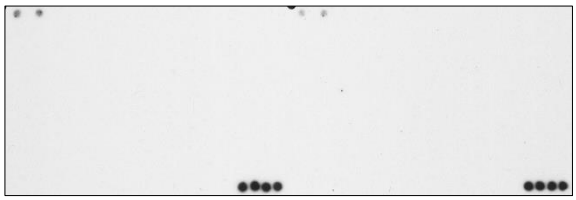


Patient 20 (30 sec)

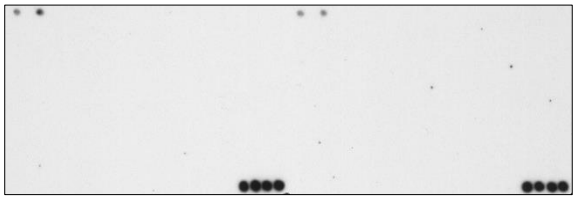


Patient 21 (30 sec)

D



Non-allergic control serum N_1 (30 sec)



Non-allergic control serum N_2 (30 sec)

Figure A21: Spotting layout and IgE immunodetection of Gly m 8 peptides using soybean-allergic, soybean-tolerant and control sera.

(A) Spotting layout of Gly m 8 multipeptide microarray. For simplification, only the left segment of the multipeptide microarray is shown. Full-length sequence of Gly m 8 is represented by 32 peptides that were spotted (each 0.04 μl) in duplicate on each segment leading to quadruplicate peptide presentation on the whole array. Biotinylated control peptides, abbreviated by a “C”, were spotted in duplicate in different dilutions (top left and bottom right on each array element) and were used as position markers. Gray empty spots represent blank spots composed of peptide printing buffer (DMSO). Blue empty spots represent internal control peptides not relevant for peptide analysis. (B) IgE immunodetection of soybean-allergic children, (C) of soybean-tolerant children and (D) of control sera. The exposure times are shown in parentheses and ranged from 15 sec to 30 sec.

7.1.5 Calculated Z-scores of patients and controls

7.1.5.1 Calculated Z-scores of Ara h 1 peptides

Table A1: Calculated Z-scores of Ara h 1 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 1 for each peanut-allergic patient (patients 1-7) are listed. Identified candidate diagnostic peptides are highlighted in light blue.

	Patient No. →	1	2	3	4	5	6	7
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	K-S-S-P-Y-Q-K-K-T-E-N-P-C-A-Q	-0.43249	-1.18966	-3.05334	-0.09238	-0.65726	7.716425	-0.86427
2	Y-Q-K-K-T-E-N-P-C-A-Q-R-C-L-Q	-0.2293	-0.97681	-2.40181	1.058556	-0.65098	8.98971	0.126512
3	T-E-N-P-C-A-Q-R-C-L-Q-S-C-Q-Q	0.153717	-0.62976	-2.5701	1.477649	-0.69201	2.972944	-0.59167
4	C-A-Q-R-C-L-Q-S-C-Q-Q-E-P-D-D	0.563348	-1.05131	-2.33272	1.088213	-0.49084	8.195964	-0.71917
5	C-L-Q-S-C-Q-Q-E-P-D-D-L-K-Q-K	1.443808	-0.28428	-1.65926	2.21333	-0.8318	1.848827	-1.26008
6	C-Q-Q-E-P-D-D-L-K-Q-K-A-C-E-S	-0.66379	2.051137	-3.33817	-0.41734	-1.50153	1.121159	-1.41863
7	P-D-D-L-K-Q-K-A-C-E-S-R-C-T-K	-0.14799	-1.12187	-2.29136	1.653279	-0.87354	4.04668	-0.69242
8	K-Q-K-A-C-E-S-R-C-T-K-L-E-Y-D	0.48681	-0.99994	-2.88187	5.476836	-0.88659	0.771805	-0.81759
9	C-E-S-R-C-T-K-L-E-Y-D-P-R-C-V	0.534612	1.138048	-1.91411	3.82531	-0.68819	0.306613	-0.65364
10	C-T-K-L-E-Y-D-P-R-C-V-Y-D-P-R	0.655743	-0.8854	-2.34763	4.414928	-1.11059	1.005971	-0.74646
11	E-Y-D-P-R-C-V-Y-D-P-R-G-H-T-G	0.634182	-0.91976	-1.6751	0.439552	-0.53513	-0.24683	-0.91261
12	R-C-V-Y-D-P-R-G-H-T-G-T-T-N-Q	0.284124	-1.60874	-2.96043	0.435003	-1.51376	6.293259	-1.44391
13	D-P-R-G-H-T-G-T-T-N-Q-R-S-P-P	2.609384	-0.6158	-1.68992	0.874976	-0.93787	1.920053	-0.29025
14	H-T-G-T-T-N-Q-R-S-P-P-G-E-R-T	1.816218	-1.21229	-2.31373	1.663906	-1.08872	3.696066	0.102456
15	T-N-Q-R-S-P-P-G-E-R-T-R-G-R-Q	1.216094	-2.00447	-2.11337	2.492032	-2.00506	6.960738	-0.93245
16	S-P-P-G-E-R-T-R-G-R-Q-P-G-D-Y	4.178911	-1.64683	-2.83959	1.789947	-1.60216	67.89797	-1.43876
17	E-R-T-R-G-R-Q-P-G-D-Y-D-D-D-R	10.12458	-1.28711	-2.23356	0.344458	-0.8324	81.6531	-0.84989
18	G-R-Q-P-G-D-Y-D-D-D-R-R-Q-P-R	0.499342	-1.14904	-2.52441	8.420034	-1.62162	-0.07544	-1.38884
19	G-D-Y-D-D-D-R-R-Q-P-R-R-E-E-G	0.645933	-0.97055	-3.05236	2.786423	-1.81955	-0.31908	-1.49194
20	D-D-R-R-Q-P-R-R-E-E-G-G-R-W-G	-0.85795	-0.57554	-3.17097	1.561276	-0.9283	3.664378	-0.76688
21	Q-P-R-R-E-E-G-G-R-W-G-P-A-G-P	-1.06165	-1.46187	-3.35184	0.753237	-1.44382	76.18538	-1.50043
22	E-E-G-G-R-W-G-P-A-G-P-R-E-R-E	-0.40662	-0.95559	-2.55054	-0.51915	-0.97002	81.88536	-0.6107
23	R-W-G-P-A-G-P-R-E-R-E-R-E-E-D	0.429307	-0.68273	-2.33809	0.260981	-0.41173	7.670801	-0.626
24	A-G-P-R-E-R-E-R-E-E-D-W-R-Q-P	-1.40584	-2.09736	-3.83454	2.92855	-1.56015	5.770623	-1.53188
25	E-R-E-R-E-E-D-W-R-Q-P-R-E-D-W	-0.71285	-0.88072	-2.60752	1.588496	-0.71774	59.90033	-0.60705
26	E-E-D-W-R-Q-P-R-E-D-W-R-R-P-S	-0.98825	-1.48975	-3.26446	-0.27369	-1.26383	60.52137	-1.21396
27	R-Q-P-R-E-D-W-R-R-P-S-H-Q-Q-P	-0.75524	-1.13707	-2.65027	0.466622	-0.96579	53.11317	-0.52431
28	E-D-W-R-R-P-S-H-Q-Q-P-R-K-I-R	4.940022	-0.99485	-0.36389	1.985738	-0.51119	78.19949	2.075901
29	R-P-S-H-Q-Q-P-R-K-I-R-P-E-G-R	2.265152	-1.04701	-1.68812	4.222064	-1.18299	28.881	0.111065
30	Q-Q-P-R-K-I-R-P-E-G-R-E-G-E-Q	1.034502	-0.75841	-1.71815	3.888645	-0.75447	38.68586	-0.30765
31	K-I-R-P-E-G-R-E-G-E-Q-E-W-G-T	-0.65872	-1.05522	-2.82396	7.031268	-1.39863	4.558378	-0.05905
32	E-G-R-E-G-E-Q-E-W-G-T-P-G-S-H	-0.06651	-0.80743	-2.30485	1.767797	-0.63977	8.899734	-0.39689
33	G-E-Q-E-W-G-T-P-G-S-H-V-R-E-E	-0.02294	-0.94347	-2.71136	2.738762	-1.23242	7.549486	-0.63765
34	W-G-T-P-G-S-H-V-R-E-E-T-S-R-N	1.628933	-0.62304	-2.10483	1.045301	-0.80641	5.973505	-0.33938
35	G-S-H-V-R-E-E-T-S-R-N-N-P-F-Y	0.636331	-0.47137	-1.82097	0.279929	-0.40971	3.96377	-0.06044
36	R-E-E-T-S-R-N-N-P-F-Y-F-P-S-R	2.021754	-1.21388	-2.28538	1.723476	-1.21498	6.878459	0.359025

37	S-R-N-N-P-F-Y-F-P-S-R-R-F-S-T	-1.61488	-3.90439	1.386388	-0.10317	-4.13888	2.741538	5.037962
38	P-F-Y-F-P-S-R-R-F-S-T-R-Y-G-N	-3.6408	-5.25917	-4.80843	0.253403	-5.55455	-1.36342	2.386002
39	P-S-R-R-F-S-T-R-Y-G-N-Q-N-G-R	0.64892	-1.69285	-0.25422	7.108303	-2.46291	-1.47748	-0.03432
40	F-S-T-R-Y-G-N-Q-N-G-R-I-R-V-L	-1.00631	-5.05172	-2.73662	14.43878	-5.58905	1.131453	-3.24344
41	Y-G-N-Q-N-G-R-I-R-V-L-Q-R-F-D	-2.5026	-4.52551	-2.23956	7.177587	-4.36578	-1.71137	-2.06414
42	N-G-R-I-R-V-L-Q-R-F-D-Q-R-S-R	-1.54719	-2.4935	0.936244	0.204262	-2.45237	1.437799	6.335763
43	R-V-L-Q-R-F-D-Q-R-S-R-Q-F-Q-N	0.271093	-0.70221	-2.83464	0.730639	-1.49754	2.598513	-1.27939
44	R-F-D-Q-R-S-R-Q-F-Q-N-L-Q-N-H	-0.34418	-0.84256	-3.47266	1.224827	-1.25758	14.0627	-0.93492
45	R-S-R-Q-F-Q-N-L-Q-N-H-R-I-V-Q	-0.94922	-2.2791	-4.09536	9.261045	-2.78771	0.6901	-0.64
46	F-Q-N-L-Q-N-H-R-I-V-Q-I-E-A-K	-1.66267	-2.39744	-4.48915	5.254972	-3.1985	-0.78143	-0.75585
47	Q-N-H-R-I-V-Q-I-E-A-K-P-N-T-L	0.321422	-0.22564	-1.83485	0.184521	-0.70741	3.47718	-0.15816
48	I-V-Q-I-E-A-K-P-N-T-L-V-L-P-K	0.795895	-0.72068	-2.32937	5.107443	-1.13325	4.954379	-0.51515
49	E-A-K-P-N-T-L-V-L-P-K-H-A-D-A	0.969916	-1.22504	-2.65041	3.547122	-1.23345	30.48927	0.175025
50	N-T-L-V-L-P-K-H-A-D-A-D-N-I-L	0.203866	-0.99771	-2.49364	2.809642	-0.67304	13.3791	-0.49408
51	L-P-K-H-A-D-A-D-N-I-L-V-I-Q-Q	0.594593	-1.6771	-2.82755	12.739	-1.78432	16.81422	-0.01487
52	A-D-A-D-N-I-L-V-I-Q-Q-G-Q-A-T	-1.32498	-2.92181	-4.15424	7.809956	-3.21609	10.47864	-1.55307
53	N-I-L-V-I-Q-Q-G-Q-A-T-V-T-V-A	0.301779	0.108303	-1.96744	3.858029	-1.20503	15.00025	0.098984
54	I-Q-Q-G-Q-A-T-V-T-V-A-N-G-N-N	1.666052	-0.89511	-2.61061	8.318539	-1.97238	6.707803	-0.39158
55	Q-A-T-V-T-V-A-N-G-N-N-R-K-S-F	-0.40526	-3.13219	-3.45844	22.60138	-3.93254	8.712123	-1.77557
56	T-V-A-N-G-N-N-R-K-S-F-N-L-D-E	2.110252	-1.19142	-2.95207	9.664301	-1.84787	15.97225	0.406638
57	G-N-N-R-K-S-F-N-L-D-E-G-H-A-L	-0.04463	-3.27968	-4.6761	16.18581	-4.00906	7.527263	-1.70687
58	K-S-F-N-L-D-E-G-H-A-L-R-I-P-S	0.951784	-0.39681	-2.24812	7.449546	-0.95312	2.657548	0.436556
59	L-D-E-G-H-A-L-R-I-P-S-G-F-I-S	0.354827	-0.84085	-0.75271	-0.33647	-0.87988	5.476149	0.185419
60	H-A-L-R-I-P-S-G-F-I-S-Y-I-L-N	1.27983	-0.34013	-1.90229	4.789845	-0.69278	3.275921	-0.19787
61	I-P-S-G-F-I-S-Y-I-L-N-R-H-D-N	-1.44875	-2.2778	-3.48475	5.888362	-2.59275	-1.22461	-0.60346
62	F-I-S-Y-I-L-N-R-H-D-N-Q-N-L-R	-0.56413	-3.99176	-3.09968	4.02033	-4.35063	2.003851	0.121354
63	I-L-N-R-H-D-N-Q-N-L-R-V-A-K-I	-0.29482	-2.41932	-4.8978	7.745597	-3.48009	-1.30032	-1.52099
64	H-D-N-Q-N-L-R-V-A-K-I-S-M-P-V	0.763553	-2.10075	-3.1747	9.112189	-2.6302	1.556026	-0.03869
65	N-L-R-V-A-K-I-S-M-P-V-N-T-P-G	1.762748	-0.65806	-1.54009	1.387809	-1.26901	4.943701	-0.14482
66	A-K-I-S-M-P-V-N-T-P-G-Q-F-E-D	0.097679	-2.00263	-2.70565	2.404973	-2.45659	3.865393	-2.0749
67	M-P-V-N-T-P-G-Q-F-E-D-F-F-P-A	2.081545	-1.66788	-3.16714	7.203564	-2.6671	-1.41603	-2.08979
68	T-P-G-Q-F-E-D-F-F-P-A-S-S-R-D	2.276861	-0.7278	-3.8584	7.067924	-2.77275	7.948578	-1.18195
69	F-E-D-F-F-P-A-S-S-R-D-Q-S-S-Y	1.174604	-3.72272	-6.29759	20.07324	-5.30457	-1.65357	-1.29931
70	F-P-A-S-S-R-D-Q-S-S-Y-L-Q-G-F	1.6363	-1.74259	-3.67252	7.208388	-2.34978	1.939091	-0.43325
71	S-R-D-Q-S-S-Y-L-Q-G-F-S-R-N-T	0.003564	-0.53398	-1.91013	2.44172	-0.73358	5.228478	0.554209
72	S-S-Y-L-Q-G-F-S-R-N-T-L-E-A-A	1.343762	-1.98017	-1.80987	6.121739	-2.19501	46.65005	-0.26445
73	Q-G-F-S-R-N-T-L-E-A-A-F-N-A-E	1.023823	-0.90585	-1.99593	2.139241	-0.94896	19.84758	-0.1404
74	R-N-T-L-E-A-A-F-N-A-E-F-N-E-I	0.487275	-0.8667	-2.31442	5.184757	-0.82155	18.61958	0.406454
75	E-A-A-F-N-A-E-F-N-E-I-R-R-V-L	-2.26439	-3.50915	-4.63532	6.326209	-3.96216	84.79687	-0.81812
76	N-A-E-F-N-E-I-R-R-V-L-L-E-E-N	-1.97503	-2.73207	-3.96148	4.349267	-3.12254	17.50704	-1.02198
77	N-E-I-R-R-V-L-L-E-E-N-A-G-G-E	1.019229	-0.27111	-0.96504	3.539903	-0.76229	4.903384	0.877217
78	R-V-L-L-E-E-N-A-G-G-E-Q-E-E-R	-0.39796	-1.09535	-2.21322	15.47838	-1.34488	2.734498	-0.15986
79	E-E-N-A-G-G-E-Q-E-E-R-G-Q-R-R	-0.27188	-2.13181	-3.10937	7.975181	-2.59953	82.8984	0.016161
80	G-G-E-Q-E-E-R-G-Q-R-R-W-S-T-R	-1.18236	-0.83977	-3.09828	4.938107	-1.36916	71.03789	-0.18467
81	E-E-R-G-Q-R-R-W-S-T-R-S-S-E-N	-0.88357	-1.12268	-2.95214	3.641478	-1.90443	1.887181	-1.37766

82	Q-R-R-W-S-T-R-S-S-E-N-N-E-G-V	0.010135	-0.57115	-2.16878	6.035965	-1.05423	3.055322	-0.5497
83	S-T-R-S-S-E-N-N-E-G-V-I-V-K-V	0.349271	-0.71599	-2.16071	0.067494	-0.6455	4.35222	-0.54183
84	S-E-N-N-E-G-V-I-V-K-V-S-K-E-H	0.619446	-0.64471	-2.0279	0.867102	-0.57462	3.853674	-0.07418
85	E-G-V-I-V-K-V-S-K-E-H-V-E-E-L	0.176544	-0.95783	-2.69025	2.578179	-1.09293	4.65444	-0.6995
86	V-K-V-S-K-E-H-V-E-E-L-T-K-H-A	1.029564	-0.73646	-1.99448	5.78152	-0.7728	3.759325	0.359229
87	K-E-H-V-E-E-L-T-K-H-A-K-S-V-S	1.60631	-0.60872	-1.65484	1.650923	-0.48078	2.590366	-0.13638
88	E-E-L-T-K-H-A-K-S-V-S-K-K-G-S	4.137314	-1.84453	-1.87632	12.86526	-3.04345	7.045041	0.083039
89	K-H-A-K-S-V-S-K-K-G-S-E-E-E-G	0.620582	-1.14283	-2.26767	0.840543	-1.05761	1.924737	-0.46654
90	S-V-S-K-K-G-S-E-E-E-G-D-I-T-N	0.943515	-1.08291	-2.35526	1.550687	-1.44285	1.899378	-0.94539
91	K-G-S-E-E-E-G-D-I-T-N-P-I-N-L	1.974438	-2.46689	-3.80354	2.726453	-3.45365	41.15605	-3.09655
92	E-E-G-D-I-T-N-P-I-N-L-R-E-G-E	0.22862	-0.62852	-3.16838	4.254572	-1.46068	79.51409	-0.95017
93	I-T-N-P-I-N-L-R-E-G-E-P-D-L-S	0.363528	-0.60529	-2.73898	1.296163	-1.11378	15.28644	-0.85971
94	I-N-L-R-E-G-E-P-D-L-S-N-N-F-G	-1.22606	-2.38749	-4.7741	5.363796	-3.81699	3.868038	-2.57686
95	E-G-E-P-D-L-S-N-N-F-G-K-L-F-E	0.69008	-0.18977	-1.78817	0.455686	-0.32037	23.86536	-0.04119
96	D-L-S-N-N-F-G-K-L-F-E-V-K-P-D	0.095137	-1.33558	-2.82432	3.57121	-1.46772	71.06849	-0.03759
97	N-F-G-K-L-F-E-V-K-P-D-K-K-N-P	0.286455	-0.33365	-1.52579	0.120074	-0.1989	87.98348	0.597717
98	L-F-E-V-K-P-D-K-K-N-P-Q-L-Q-D	0.985374	-0.23446	-1.40196	0.416865	-0.5247	33.65645	-0.05545
99	K-P-D-K-K-N-P-Q-L-Q-D-L-D-M-M	-0.29081	-0.96209	-2.08834	1.909313	-0.52197	34.5097	-0.05699
100	K-N-P-Q-L-Q-D-L-D-M-M-L-T-C-V	-0.23861	-0.8442	-1.73651	0.38165	-0.62912	11.72243	-0.43121
101	L-Q-D-L-D-M-M-L-T-C-V-E-I-K-E	0.065326	-0.2387	-1.04457	1.169097	-0.49752	1.17193	-0.1127
102	D-M-M-L-T-C-V-E-I-K-E-G-A-L-M	0.52351	0.005215	-1.46727	0.730989	-0.52578	7.882506	0.078658
103	T-C-V-E-I-K-E-G-A-L-M-L-P-H-F	0.390627	-0.4551	-1.47642	0.445399	-0.25364	10.56739	-0.10895
104	I-K-E-G-A-L-M-L-P-H-F-N-S-K-A	0.678282	-0.24142	-1.89884	1.928522	-0.34137	5.38557	0.928582
105	A-L-M-L-P-H-F-N-S-K-A-M-V-I-V	1.10833	-0.59734	-1.70139	0.535054	-0.63438	14.95519	0.626058
106	P-H-F-N-S-K-A-M-V-I-V-V-V-N-K	0.224611	-0.55107	-1.52634	0.202799	-0.70191	0.916232	0.094881
107	S-K-A-M-V-I-V-V-V-N-K-G-T-G-N	0.929518	-0.83905	-1.78553	-0.42105	-0.58485	7.496838	-0.50099
108	V-I-V-V-V-N-K-G-T-G-N-L-E-L-V	1.103849	0.010972	-1.53444	0.860202	-0.21427	2.499031	0.296273
109	V-N-K-G-T-G-N-L-E-L-V-A-V-R-K	1.476227	-0.3665	-1.61933	-0.15819	-0.90177	5.014058	-0.41616
110	T-G-N-L-E-L-V-A-V-R-K-E-Q-Q-Q	0.812081	-1.00644	-2.01111	1.745129	-0.83667	8.070343	-0.01603
111	E-L-V-A-V-R-K-E-Q-Q-Q-R-G-R-R	0.306754	-1.29848	-0.50695	1.555976	-1.61355	22.57317	0.803256
112	V-R-K-E-Q-Q-Q-R-G-R-R-E-E-E-E	0.078815	-0.80032	-2.77954	4.94899	-1.70217	3.583848	-1.90107
113	Q-Q-Q-R-G-R-R-E-E-E-E-D-E-D-E	1.158755	-0.4896	-1.79243	2.329585	-0.96563	0.448798	-0.30257
114	G-R-R-E-E-E-E-D-E-D-E-E-E-E-G	0.150689	-0.81422	-2.54762	-0.08744	-1.45023	-0.96502	-1.56727
115	E-E-E-D-E-D-E-E-E-E-G-S-N-R-E	1.672738	-1.59803	-3.12167	3.3619	-2.04558	-1.28224	-1.93551
116	E-D-E-E-E-E-G-S-N-R-E-V-R-R-Y	-1.16297	-2.06335	-4.02093	1.705248	-2.59788	3.063667	-1.50236
117	E-E-G-S-N-R-E-V-R-R-Y-T-A-R-L	-0.75254	-1.55481	-3.60538	0.118237	-2.69302	5.949141	0.456818
118	N-R-E-V-R-R-Y-T-A-R-L-K-E-G-D	-1.92596	-2.85343	-4.8028	-0.62052	-4.11738	10.09974	-1.58336
119	R-R-Y-T-A-R-L-K-E-G-D-V-F-I-M	-1.76455	-2.34967	-3.61761	1.666245	-2.62181	12.91177	-0.40054
120	A-R-L-K-E-G-D-V-F-I-M-P-A-A-H	-0.18742	-0.66667	-1.92492	1.311712	-0.47342	8.499678	-0.16121
121	E-G-D-V-F-I-M-P-A-A-H-P-V-A-I	0.34946	-0.50288	-1.66923	-0.0182	-0.36326	13.05531	0.204164
122	F-I-M-P-A-A-H-P-V-A-I-N-A-S-S	1.25892	-0.60374	-1.87249	-0.73392	-0.67999	17.804	-0.61094
123	A-A-H-P-V-A-I-N-A-S-S-E-L-H-L	1.245033	-0.29496	-1.47561	0.488373	-0.39192	9.969459	-0.27587
124	V-A-I-N-A-S-S-E-L-H-L-L-G-F-G	0.905583	-0.35988	-2.10398	1.256405	-0.9979	2.617616	-0.51217
125	A-S-S-E-L-H-L-L-G-F-G-I-N-A-E	0.721812	-0.68413	-0.73892	5.691862	-0.93617	1.93768	0.801827
126	L-H-L-L-G-F-G-I-N-A-E-N-N-H-R	-0.18883	-0.35492	-1.08954	1.70115	-0.94341	1.156267	2.945872

127	G-F-G-I-N-A-E-N-N-H-R-I-F-L-A	0.859069	-1.03097	-1.37157	4.770907	-1.28904	5.671551	1.244614
128	N-A-E-N-N-H-R-I-F-L-A-G-D-K-D	1.811148	-0.05654	-1.71842	1.193345	-0.89886	41.52316	0.340664
129	N-H-R-I-F-L-A-G-D-K-D-N-V-I-D	0.689382	-0.93498	-2.56878	6.038987	-1.64294	34.07875	-0.12066
130	F-L-A-G-D-K-D-N-V-I-D-Q-I-E-K	-0.86229	-1.16338	-3.08074	1.12141	-2.24074	3.813532	-1.53249
131	D-K-D-N-V-I-D-Q-I-E-K-Q-A-K-D	0.601287	-0.84525	-2.34227	0.4441	-1.01161	3.196559	-0.2558
132	V-I-D-Q-I-E-K-Q-A-K-D-L-A-F-P	0.426946	0.2044	-1.31044	0.142505	-0.10134	12.89432	-0.04939
133	I-E-K-Q-A-K-D-L-A-F-P-G-S-G-E	1.395817	-0.13212	-1.37324	0.28643	-0.41136	32.46964	-0.20266
134	A-K-D-L-A-F-P-G-S-G-E-Q-V-E-K	4.439058	0.345446	-1.45834	0.167279	-0.44265	56.61579	-0.31642
135	A-F-P-G-S-G-E-Q-V-E-K-L-I-K-N	1.561207	-0.57819	-1.84461	-0.16999	-1.69778	8.622022	-1.04232
136	S-G-E-Q-V-E-K-L-I-K-N-Q-K-E-S	0.727859	-0.76574	-1.89778	2.558091	-1.46236	10.53836	-0.67414
137	V-E-K-L-I-K-N-Q-K-E-S-H-F-V-S	1.527385	-1.00239	-2.06928	1.337903	-1.4349	2.36331	-1.27941
138	I-K-N-Q-K-E-S-H-F-V-S-A-R-P-Q	1.403968	-1.02841	-2.32454	1.391209	-1.3102	6.293002	-0.49962
139	K-E-S-H-F-V-S-A-R-P-Q-S-Q-S-Q	0.653673	-0.58107	-2.68351	-0.61288	-0.9825	2.940888	-0.73043
140	F-V-S-A-R-P-Q-S-Q-S-Q-S-P-S-S	1.62329	-0.69575	-2.2226	0.772396	-1.25317	6.354181	-0.86096
141	R-P-Q-S-Q-S-Q-S-P-S-S-P-E-K-E	1.29013	-3.78136	-5.68413	8.070539	-5.28954	0.126745	-3.70254
142	Q-S-Q-S-P-S-S-P-E-K-E-S-P-E-K	-0.16997	-1.35748	-3.23488	1.399295	-2.44152	5.067135	-0.3413
143	P-S-S-P-E-K-E-S-P-E-K-E-D-Q-E	-0.4096	-0.66911	-2.1105	1.671941	-1.1692	1.26978	0.644171
144	E-K-E-S-P-E-K-E-D-Q-E-E-E-N-Q	-0.14961	-0.4581	-1.50818	1.138381	-0.75974	0.698105	-0.5293
145	P-E-K-E-D-Q-E-E-E-N-Q-G-G-K-G	0.106742	-0.36948	-1.19924	-0.40625	-0.38914	5.165898	-0.05509
146	D-Q-E-E-E-N-Q-G-G-K-G-P-L-L-S	0.560885	-0.68346	-1.61258	1.074246	-0.65625	11.72104	-0.29255
147	E-N-Q-G-G-K-G-P-L-L-S-I-L-K-A	-0.90939	-1.30346	-1.1399	0.047233	-1.83448	13.87053	0.385225
148	Q-G-G-K-G-P-L-L-S-I-L-K-A-F-N	0.456469	-0.45187	-1.2362	1.783823	-0.87363	3.187916	0.243678
	m (blanks)	12892.3	12559.93	13066.22	11346.16	12750	12111.35	13386.11
	s (blanks)	431.2739	445.7373	676.0718	511.396	505.1061	521.3369	590.8274

Table A2: Calculated Z-scores of Ara h 1 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 1 for each peanut-allergic patient (patients 8-14) are listed. Identified candidate diagnostic peptides are highlighted in light blue.

	Patient No. →	8	9	10	11	12	13	14
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	K-S-S-P-Y-Q-K-K-T-E-N-P-C-A-Q	-1.95659	-0.9748	28.32213	-1.02056	3.154055	-1.3093	-0.95876
2	Y-Q-K-K-T-E-N-P-C-A-Q-R-C-L-Q	-1.70399	-0.72228	27.98061	-0.96722	15.07417	-1.29529	-0.54623
3	T-E-N-P-C-A-Q-R-C-L-Q-S-C-Q-Q	-1.90137	-0.92278	30.25642	-1.32067	5.405221	-1.42	-0.41925
4	C-A-Q-R-C-L-Q-S-C-Q-Q-E-P-D-D	-2.22736	-0.88858	24.55488	-0.74194	8.777184	-1.15074	-0.16411
5	C-L-Q-S-C-Q-Q-E-P-D-D-L-K-Q-K	-2.68886	-1.13065	30.47329	-1.04345	54.6107	-1.62027	-0.20494
6	C-Q-Q-E-P-D-D-L-K-Q-K-A-C-E-S	-2.96386	-1.53973	4.569288	-1.99576	36.5301	-2.26513	-1.21718
7	P-D-D-L-K-Q-K-A-C-E-S-R-C-T-K	-3.06115	-1.21949	29.04902	-1.44213	4.512134	-1.74131	-0.30216
8	K-Q-K-A-C-E-S-R-C-T-K-L-E-Y-D	-2.50033	-1.02714	15.88542	-1.16613	3.087929	-1.6131	-0.25352
9	C-E-S-R-C-T-K-L-E-Y-D-P-R-C-V	-2.0122	-0.63176	9.339628	-1.05525	1.836523	-1.48725	0.04713
10	C-T-K-L-E-Y-D-P-R-C-V-Y-D-P-R	-3.13689	-0.90988	24.98933	-1.43512	4.969902	-1.79121	-0.24999
11	E-Y-D-P-R-C-V-Y-D-P-R-G-H-T-G	-1.65627	-0.76646	4.96319	-0.95284	0.437485	-1.46187	0.035702
12	R-C-V-Y-D-P-R-G-H-T-G-T-T-N-Q	-3.76779	-1.40395	20.80713	-1.86595	8.541282	-2.18965	-0.79305
13	D-P-R-G-H-T-G-T-T-N-Q-R-S-P-P	-2.72852	-1.01334	32.48072	-1.19182	20.23727	-1.89416	-0.11027
14	H-T-G-T-T-N-Q-R-S-P-P-G-E-R-T	-3.04365	-1.14844	20.47111	-1.67933	27.20273	-1.8419	-0.14598

15	T-N-Q-R-S-P-P-G-E-R-T-R-G-R-Q	-4.35739	-1.76217	31.43215	-2.22998	67.17763	-2.5868	-1.03452
16	S-P-P-G-E-R-T-R-G-R-Q-P-G-D-Y	-4.60482	-1.61098	43.95711	-2.17648	40.16761	-2.47917	-0.43438
17	E-R-T-R-G-R-Q-P-G-D-Y-D-D-D-R	-4.27141	-0.89645	44.84097	-1.75723	62.55321	-1.8174	0.151566
18	G-R-Q-P-G-D-Y-D-D-D-R-R-Q-P-R	-4.92826	-2.26625	40.27803	-2.31852	2.922465	-3.20201	-1.0525
19	G-D-Y-D-D-D-R-R-Q-P-R-R-E-E-G	-4.30468	-2.01768	33.4155	-1.56813	4.812093	-2.24927	-2.15071
20	D-D-R-R-Q-P-R-R-E-E-G-G-R-W-G	-3.84646	-0.77439	40.84421	-0.79981	4.347127	-1.15198	-1.63586
21	Q-P-R-R-E-E-G-G-R-W-G-P-A-G-P	-4.08243	-1.30404	39.99965	-1.09358	42.02121	-1.68646	-1.57333
22	E-E-G-G-R-W-G-P-A-G-P-R-E-R-E	-3.20261	-0.9118	38.09706	-0.81459	56.07108	-1.31945	-1.10252
23	R-W-G-P-A-G-P-R-E-R-E-R-E-E-D	-2.1785	-0.13587	28.48982	-0.83454	10.54478	-0.82855	-1.06853
24	A-G-P-R-E-R-E-R-E-E-D-W-R-Q-P	-4.01635	-1.67219	27.05348	-1.36109	11.67284	-2.45914	-2.01368
25	E-R-E-R-E-E-D-W-R-Q-P-R-E-D-W	-2.34874	-0.74291	20.3634	-0.81399	13.07171	-1.07726	-0.63446
26	E-E-D-W-R-Q-P-R-E-D-W-R-R-P-S	-2.43097	-1.25448	31.45863	-1.69233	56.73027	-1.71155	-1.18392
27	R-Q-P-R-E-D-W-R-R-P-S-H-Q-Q-P	-2.42284	-0.88603	27.67624	-1.34498	48.56553	-1.37356	-1.07931
28	E-D-W-R-R-P-S-H-Q-Q-P-R-K-I-R	-1.7439	-0.75816	39.15998	-0.84611	51.25444	-0.98538	-0.02562
29	R-P-S-H-Q-Q-P-R-K-I-R-P-E-G-R	-1.55411	-1.34788	41.66455	-1.2656	64.91187	-1.60245	-0.11078
30	Q-Q-P-R-K-I-R-P-E-G-R-E-G-E-Q	-2.41992	-0.74356	40.1034	-1.11412	26.24605	-1.55143	-0.56376
31	K-I-R-P-E-G-R-E-G-E-Q-E-W-G-T	-3.6755	-1.47951	26.54775	-1.52008	6.79105	-1.6782	-0.47748
32	E-G-R-E-G-E-Q-E-W-G-T-P-G-S-H	-3.22131	-0.75038	41.81443	-0.97398	64.54864	-1.03388	-0.18995
33	G-E-Q-E-W-G-T-P-G-S-H-V-R-E-E	-3.49618	-1.16457	34.43459	-1.58677	32.8207	-1.75933	-0.39217
34	W-G-T-P-G-S-H-V-R-E-E-T-S-R-N	-2.78312	-0.70042	31.02987	-1.14687	12.60519	-1.37668	-0.2298
35	G-S-H-V-R-E-E-T-S-R-N-N-P-F-Y	-1.88367	-0.39236	24.06783	-0.7242	7.009681	-0.87195	0.118452
36	R-E-E-T-S-R-N-N-P-F-Y-F-P-S-R	-3.04226	-1.28867	28.31891	-1.42375	38.86193	-1.70589	-0.51971
37	S-R-N-N-P-F-Y-F-P-S-R-R-F-S-T	-5.26766	-4.13936	19.39534	-4.62836	44.0612	-4.31022	-3.28579
38	P-F-Y-F-P-S-R-R-F-S-T-R-Y-G-N	-6.76174	-5.29454	-1.0646	-5.86614	42.61004	-6.57917	-4.86849
39	P-S-R-R-F-S-T-R-Y-G-N-Q-N-G-R	-5.01532	-2.61147	11.58551	-2.51812	66.81428	-3.15281	-0.97199
40	F-S-T-R-Y-G-N-Q-N-G-R-I-R-V-L	-9.74517	-5.95433	23.02381	-6.23201	8.94139	-6.80864	-3.89949
41	Y-G-N-Q-N-G-R-I-R-V-L-Q-R-F-D	-7.63284	-4.66225	8.338671	-5.01255	3.68042	-5.58776	-3.2951
42	N-G-R-I-R-V-L-Q-R-F-D-Q-R-S-R	-4.33543	-2.67799	8.863296	-3.26766	-0.76943	-3.54223	-1.45271
43	R-V-L-Q-R-F-D-Q-R-S-R-Q-F-Q-N	-3.67881	-1.49484	31.49582	-1.6495	4.079253	-1.77369	-2.56039
44	R-F-D-Q-R-S-R-Q-F-Q-N-L-Q-N-H	-3.54523	-1.20429	38.92599	-1.0706	11.6329	-1.93416	-2.38623
45	R-S-R-Q-F-Q-N-L-Q-N-H-R-I-V-Q	-4.28232	-2.5037	21.27771	-2.60691	2.570334	-2.92144	-2.43714
46	F-Q-N-L-Q-N-H-R-I-V-Q-I-E-A-K	-4.67516	-3.37761	10.424	-2.14983	6.189928	-3.43808	-2.45735
47	Q-N-H-R-I-V-Q-I-E-A-K-P-N-T-L	-2.08582	-0.39765	26.67359	-0.78134	29.03791	-0.8685	-1.22068
48	I-V-Q-I-E-A-K-P-N-T-L-V-L-P-K	-2.72193	-1.26749	29.14652	-0.23567	36.90959	-1.22451	-1.32635
49	E-A-K-P-N-T-L-V-L-P-K-H-A-D-A	-2.40047	-1.03821	38.93817	-1.28687	37.0244	-1.53828	-1.55528
50	N-T-L-V-L-P-K-H-A-D-A-D-N-I-L	-2.30965	-0.88346	21.02735	-1.08714	11.17291	-1.50525	-0.8377
51	L-P-K-H-A-D-A-D-N-I-L-V-I-Q-Q	-3.06083	-1.75711	26.34838	-1.69477	11.04157	-1.99793	-1.41468
52	A-D-A-D-N-I-L-V-I-Q-Q-G-Q-A-T	-4.32171	-3.35	31.99103	-2.46565	8.986276	-3.03584	-2.08664
53	N-I-L-V-I-Q-Q-G-Q-A-T-V-T-V-A	-2.71195	-0.88165	31.87971	-1.18715	26.16084	-1.77105	-0.70725
54	I-Q-Q-G-Q-A-T-V-T-V-A-N-G-N-N	-4.36631	-2.03965	36.56951	-1.28325	8.660403	-2.52724	-1.57681
55	Q-A-T-V-T-V-A-N-G-N-N-R-K-S-F	-6.15323	-3.8656	31.65103	-3.26051	8.065576	-4.49434	-3.39188
56	T-V-A-N-G-N-N-R-K-S-F-N-L-D-E	-3.93427	-2.02134	38.35496	-1.80216	32.68206	-2.21638	-0.6439
57	G-N-N-R-K-S-F-N-L-D-E-G-H-A-L	-6.24716	-3.5042	21.3057	-3.82783	7.128677	-4.47682	-1.90784
58	K-S-F-N-L-D-E-G-H-A-L-R-I-P-S	-3.42325	-0.8617	14.97481	-0.98083	12.95737	-1.28267	-0.08034
59	L-D-E-G-H-A-L-R-I-P-S-G-F-I-S	-2.82814	-0.90822	17.84863	-1.13912	16.41646	-1.16782	-0.01704

60	H-A-L-R-I-P-S-G-F-I-S-Y-I-L-N	-3.03754	-0.89264	11.49409	-0.88715	10.95521	-1.27717	-0.18422
61	I-P-S-G-F-I-S-Y-I-L-N-R-H-D-N	-5.26312	-2.50612	4.403284	-2.8269	10.44105	-3.06633	-1.90367
62	F-I-S-Y-I-L-N-R-H-D-N-Q-N-L-R	-6.55293	-4.33353	7.972951	-4.43163	20.7229	-5.02993	-3.62711
63	I-L-N-R-H-D-N-Q-N-L-R-V-A-K-I	-6.56412	-3.93581	23.73727	-3.55423	10.24125	-4.14117	-2.6765
64	H-D-N-Q-N-L-R-V-A-K-I-S-M-P-V	-6.97025	-3.12334	14.49116	-2.98602	9.581601	-3.26115	-1.23369
65	N-L-R-V-A-K-I-S-M-P-V-N-T-P-G	-4.32774	-1.44849	24.12757	-1.63928	10.58732	-1.66871	-0.25848
66	A-K-I-S-M-P-V-N-T-P-G-Q-F-E-D	-5.79621	-2.9769	17.66403	-3.03783	5.944445	-3.59615	-1.07006
67	M-P-V-N-T-P-G-Q-F-E-D-F-F-P-A	-5.57132	-3.06898	4.990334	-2.07883	-1.90449	-3.01951	-4.14832
68	T-P-G-Q-F-E-D-F-F-P-A-S-S-R-D	-4.90563	-2.56269	28.01848	-1.57662	46.17325	-2.92654	-3.37069
69	F-E-D-F-F-P-A-S-S-R-D-Q-S-S-Y	-8.26829	-5.83556	18.40411	-6.03988	20.54574	-6.32858	-6.45383
70	F-P-A-S-S-R-D-Q-S-S-Y-L-Q-G-F	-4.78177	-2.05902	34.02838	-1.52097	35.56388	-2.60334	-1.49791
71	S-R-D-Q-S-S-Y-L-Q-G-F-S-R-N-T	-2.43575	-0.64598	27.28941	-0.92118	69.60847	-1.26575	-1.35977
72	S-S-Y-L-Q-G-F-S-R-N-T-L-E-A-A	-3.76234	-2.12827	39.24736	-2.39171	68.22886	-2.88902	-2.68849
73	Q-G-F-S-R-N-T-L-E-A-A-F-N-A-E	-2.30039	-0.71307	18.39498	-0.95202	61.99179	-1.31067	-1.43398
74	R-N-T-L-E-A-A-F-N-A-E-F-N-E-I	-2.56312	-0.92668	16.1269	-0.97779	29.32206	-1.19154	-0.84236
75	E-A-A-F-N-A-E-F-N-E-I-R-R-V-L	-5.58834	-4.00334	15.92522	-4.17499	60.79135	-4.24091	-3.95547
76	N-A-E-F-N-E-I-R-R-V-L-L-E-E-N	-5.47853	-3.47741	15.02592	-3.13435	26.7283	-3.56129	-2.6173
77	N-E-I-R-R-V-L-L-E-E-N-A-G-G-E	-2.10309	-1.19695	20.36413	-0.62934	7.936962	-1.13886	-0.02144
78	R-V-L-L-E-E-N-A-G-G-E-Q-E-E-R	-3.67445	-1.46928	14.95988	-1.40336	1.582423	-2.09503	-0.47297
79	E-E-N-A-G-G-E-Q-E-E-R-G-Q-R-R	-4.90388	-2.75702	20.55115	-2.39546	8.796237	-3.02114	-2.12181
80	G-G-E-Q-E-E-R-G-Q-R-R-W-S-T-R	-4.41715	-1.09659	18.1262	-1.34526	62.41933	-1.61712	-1.04978
81	E-E-R-G-Q-R-R-W-S-T-R-S-S-E-N	-4.20101	-1.67343	22.86455	-2.03128	57.77568	-1.93389	-0.78342
82	Q-R-R-W-S-T-R-S-S-E-N-N-E-G-V	-3.97633	-0.92561	17.6941	-0.59024	13.55107	-1.24286	-0.13821
83	S-T-R-S-S-E-N-N-E-G-V-I-V-K-V	-2.83874	-0.58951	18.41104	-0.88999	9.166118	-1.09391	0.073327
84	S-E-N-N-E-G-V-I-V-K-V-S-K-E-H	-2.39698	-0.53751	13.92797	-0.81565	9.493182	-1.00714	0.017886
85	E-G-V-I-V-K-V-S-K-E-H-V-E-E-L	-3.83701	-1.16178	7.494784	-1.35485	16.82802	-1.80667	-0.66811
86	V-K-V-S-K-E-H-V-E-E-L-T-K-H-A	-4.26815	-0.98531	15.01437	-0.92839	68.73451	-1.35001	-0.3372
87	K-E-H-V-E-E-L-T-K-H-A-K-S-V-S	-3.39306	-0.75574	14.43885	-0.79939	21.02147	-1.13556	0.243638
88	E-E-L-T-K-H-A-K-S-V-S-K-K-G-S	-5.93593	-2.81133	11.59875	-3.22317	17.22047	-3.54828	-1.6943
89	K-H-A-K-S-V-S-K-K-G-S-E-E-E-G	-4.13459	-1.55797	4.91766	-1.72304	0.923406	-1.83939	0.16838
90	S-V-S-K-K-G-S-E-E-E-G-D-I-T-N	-4.46371	-1.63945	11.7039	-1.36741	5.777961	-1.97402	-0.50117
91	K-G-S-E-E-E-G-D-I-T-N-P-I-N-L	-6.37508	-2.9604	29.18696	-3.41948	7.741442	-3.54009	-4.81018
92	E-E-G-D-I-T-N-P-I-N-L-R-E-G-E	-4.33165	-1.3087	23.90777	-1.21587	51.32941	-1.94206	-2.74132
93	I-T-N-P-I-N-L-R-E-G-E-P-D-L-S	-3.70798	-0.98746	37.48644	-1.3126	14.57472	-1.46316	-1.79034
94	I-N-L-R-E-G-E-P-D-L-S-N-N-F-G	-6.54988	-4.28538	30.71264	-3.89925	9.437336	-4.58642	-4.3245
95	E-G-E-P-D-L-S-N-N-F-G-K-L-F-E	-1.46864	-0.18839	28.65561	-0.62818	36.15118	-0.6046	-1.05615
96	D-L-S-N-N-F-G-K-L-F-E-V-K-P-D	-3.32656	-1.4513	17.49017	-1.81756	68.98493	-1.91991	-2.17652
97	N-F-G-K-L-F-E-V-K-P-D-K-K-N-P	-1.34616	-0.24934	43.79128	-0.40151	71.75407	-0.36747	-1.09402
98	L-F-E-V-K-P-D-K-K-N-P-Q-L-Q-D	-1.88738	-0.31497	25.41027	-0.19021	49.6988	-0.51912	-0.68788
99	K-P-D-K-K-N-P-Q-L-Q-D-L-D-M-M	-3.34328	-0.83561	22.1567	-0.72622	22.46037	-0.97099	-0.99947
100	K-N-P-Q-L-Q-D-L-D-M-M-L-T-C-V	-3.00073	-0.93314	32.93555	-0.69971	14.14359	-0.83271	-0.80638
101	L-Q-D-L-D-M-M-L-T-C-V-E-I-K-E	-2.16061	-0.75852	18.61924	-0.6068	8.41766	-0.90694	0.00928
102	D-M-M-L-T-C-V-E-I-K-E-G-A-L-M	-1.75093	-0.52462	35.61738	-0.57673	12.83006	-1.01601	-0.25256
103	T-C-V-E-I-K-E-G-A-L-M-L-P-H-F	-2.06488	-0.45057	30.74046	-0.55748	9.895376	-0.77067	-0.17947
104	I-K-E-G-A-L-M-L-P-H-F-N-S-K-A	-2.68508	-0.61064	16.36029	-0.43815	11.84385	-0.79197	-0.31379

105	A-L-M-L-P-H-F-N-S-K-A-M-V-I-V	-1.95496	-0.63669	27.3953	-0.99294	33.78562	-1.02833	-0.58665
106	P-H-F-N-S-K-A-M-V-I-V-V-V-N-K	-1.95991	-0.45497	3.332093	-0.60686	4.327043	-0.67126	-0.05001
107	S-K-A-M-V-I-V-V-V-N-K-G-T-G-N	-2.34644	-0.68172	22.68506	-0.88156	21.24308	-1.07402	-0.34188
108	V-I-V-V-V-N-K-G-T-G-N-L-E-L-V	-1.63305	-0.46442	20.19798	-0.57221	12.88336	-0.65924	0.154055
109	V-N-K-G-T-G-N-L-E-L-V-A-V-R-K	-2.61686	-0.78952	12.16114	-0.93539	14.26844	-1.21454	-0.09956
110	T-G-N-L-E-L-V-A-V-R-K-E-Q-Q-Q	-3.4309	-1.03309	22.7292	-1.01179	28.42642	-1.34545	-0.49917
111	E-L-V-A-V-R-K-E-Q-Q-Q-R-G-R-R	-4.28453	-1.46861	9.242728	-1.90113	15.69295	-1.89389	-1.03123
112	V-R-K-E-Q-Q-Q-R-G-R-R-E-E-E-E	-5.42357	-2.23325	3.134943	-2.15699	5.698975	-3.14783	-1.2912
113	Q-Q-Q-R-G-R-R-E-E-E-E-D-E-D-E	-4.28854	-1.31728	11.02947	-1.10987	2.351958	-1.79373	0.007391
114	G-R-R-E-E-E-E-D-E-D-E-E-E-E-G	-4.44486	-1.6407	5.843318	-1.54489	0.559364	-2.0747	0.238667
115	E-E-E-D-E-D-E-E-E-E-G-S-N-R-E	-5.19245	-1.75737	13.16345	-1.48821	0.230413	-1.85686	-3.66614
116	E-D-E-E-E-E-G-S-N-R-E-V-R-R-Y	-5.97016	-2.66847	26.06144	-2.5327	2.02125	-3.18929	-4.57536
117	E-E-G-S-N-R-E-V-R-R-Y-T-A-R-L	-5.46785	-2.66487	36.82831	-2.73539	8.177685	-2.67505	-3.19838
118	N-R-E-V-R-R-Y-T-A-R-L-K-E-G-D	-6.1529	-4.11179	33.40749	-3.81633	28.26598	-4.74945	-4.95809
119	R-R-Y-T-A-R-L-K-E-G-D-V-F-I-M	-4.73953	-2.52389	26.24127	-2.65123	21.76096	-3.10391	-3.59705
120	A-R-L-K-E-G-D-V-F-I-M-P-A-A-H	-2.40236	-0.68516	36.23816	-0.82042	69.34375	-0.66402	-1.4244
121	E-G-D-V-F-I-M-P-A-A-H-P-V-A-I	-1.98684	-0.5432	29.86549	-0.47905	68.33136	-0.68841	-0.91844
122	F-I-M-P-A-A-H-P-V-A-I-N-A-S-S	-2.03051	-0.81399	35.63426	-0.91636	26.31066	-0.92685	-1.04279
123	A-A-H-P-V-A-I-N-A-S-S-E-L-H-L	-1.90041	-0.4741	32.66196	-0.4481	26.87225	-0.80488	-0.41288
124	V-A-I-N-A-S-S-E-L-H-L-L-G-F-G	-2.77697	-0.9404	41.33419	-1.1193	62.21924	-1.39702	-0.58982
125	A-S-S-E-L-H-L-L-G-F-G-I-N-A-E	-2.83207	-1.36229	44.24118	-0.32327	68.95564	-1.03879	-1.0403
126	L-H-L-L-G-F-G-I-N-A-E-N-N-H-R	-1.74127	-1.19878	32.87419	-1.18007	26.69934	-1.1841	-0.98672
127	G-F-G-I-N-A-E-N-N-H-R-I-F-L-A	-2.65212	-1.02887	17.48057	-0.67573	15.64811	-1.17244	-0.71563
128	N-A-E-N-N-H-R-I-F-L-A-G-D-K-D	-2.45861	-0.82349	38.11183	-0.57664	33.53718	-1.11492	-0.77901
129	N-H-R-I-F-L-A-G-D-K-D-N-V-I-D	-3.07595	-1.49049	25.4557	-1.26836	15.31328	-1.87731	-1.27826
130	F-L-A-G-D-K-D-N-V-I-D-Q-I-E-K	-3.75404	-1.73834	14.19805	-1.85314	16.94452	-2.1449	-1.53697
131	D-K-D-N-V-I-D-Q-I-E-K-Q-A-K-D	-3.02897	-1.04999	9.62185	-0.80391	67.30114	-1.37824	-0.7272
132	V-I-D-Q-I-E-K-Q-A-K-D-L-A-F-P	-2.00311	-0.1268	16.79361	-0.21957	10.28472	-0.45562	0.350361
133	I-E-K-Q-A-K-D-L-A-F-P-G-S-G-E	-1.94985	-0.34616	19.72019	-0.39259	17.80648	-0.73441	0.028981
134	A-K-D-L-A-F-P-G-S-G-E-Q-V-E-K	-2.55642	-0.45477	39.2377	-0.48013	70.46387	-0.69394	-0.14828
135	A-F-P-G-S-G-E-Q-V-E-K-L-I-K-N	-3.97308	-1.49994	21.19931	-1.74397	60.38143	-2.01956	-0.51783
136	S-G-E-Q-V-E-K-L-I-K-N-Q-K-E-S	-3.9641	-1.57241	21.44991	-1.46967	13.61802	-1.87466	-0.40943
137	V-E-K-L-I-K-N-Q-K-E-S-H-F-V-S	-4.40277	-1.68464	23.55864	-1.54123	12.12174	-2.20248	-0.22919
138	I-K-N-Q-K-E-S-H-F-V-S-A-R-P-Q	-4.23144	-2.0294	18.97533	-1.44276	2.129672	-2.1406	-0.88678
139	K-E-S-H-F-V-S-A-R-P-Q-S-Q-S-Q	-3.49273	-0.72007	29.49061	-0.90466	3.473776	-0.87857	-2.35523
140	F-V-S-A-R-P-Q-S-Q-S-Q-S-P-S-S	-3.95662	-1.28279	34.0999	-0.10611	9.606716	-1.1403	-2.93057
141	R-P-Q-S-Q-S-Q-S-P-S-S-P-E-K-E	-8.85993	-6.37182	17.50627	-5.97246	-0.31476	-5.48508	-7.30835
142	Q-S-Q-S-P-S-S-P-E-K-E-S-P-E-K	-4.40123	-2.39583	24.36413	-1.48408	4.115392	-2.28942	-2.64742
143	P-S-S-P-E-K-E-S-P-E-K-E-D-Q-E	-2.63767	-0.93368	9.101563	-0.86804	2.84826	-1.25326	-1.30512
144	E-K-E-S-P-E-K-E-D-Q-E-E-E-N-Q	-2.69051	-0.74105	6.020583	-0.53462	5.785766	-0.82766	-1.586
145	P-E-K-E-D-Q-E-E-E-N-Q-G-G-K-G	-2.06127	-0.52956	8.681872	-0.59942	13.28093	-0.58481	-1.36498
146	D-Q-E-E-E-N-Q-G-G-K-G-P-L-L-S	-2.60921	-1.17338	22.88251	-1.05474	17.46192	-1.03748	-1.39874
147	E-N-Q-G-G-K-G-P-L-L-S-I-L-K-A	-4.25013	-1.7635	28.03346	-1.66148	15.51753	-2.11536	-2.26971
148	Q-G-G-K-G-P-L-L-S-I-L-K-A-F-N	-3.64914	-0.99271	22.94198	-0.51518	14.53463	-1.07225	-1.21109
	m (blanks)	20519.35	13674.02	11670.57	11374.72	11569.74	12656.75	11931.75

s (blanks)	805.9902	491.9029	1013.109	509.6116	658.8546	490.372	397.9312

Table A3: Calculated Z-scores of Ara h 1 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 1 for each peanut-allergic patient (patients 15-21) are listed. Identified candidate diagnostic peptides are highlighted in light blue.

	Patient No. →	15	16	17	18	19	20	21
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	K-S-S-P-Y-Q-K-K-T-E-N-P-C-A-Q	0.758263	-1.66117	8.686188	14.59167	-1.79666	-1.9845	14.8168
2	Y-Q-K-K-T-E-N-P-C-A-Q-R-C-L-Q	6.650542	-1.59004	14.27505	50.21214	-1.56814	-1.99411	18.17452
3	T-E-N-P-C-A-Q-R-C-L-Q-S-C-Q-Q	5.617234	-1.67897	7.590709	79.50092	-1.67817	-1.94595	6.551018
4	C-A-Q-R-C-L-Q-S-C-Q-Q-E-P-D-D	1.000938	-0.92755	10.06951	31.98972	-1.41009	-1.36392	17.70244
5	C-L-Q-S-C-Q-Q-E-P-D-D-L-K-Q-K	0.812902	-1.12838	11.98374	19.47551	-1.89848	-1.86399	10.61772
6	C-Q-Q-E-P-D-D-L-K-Q-K-A-C-E-S	-1.58772	-2.00222	7.512719	26.87851	-1.89182	-1.54865	16.95777
7	P-D-D-L-K-Q-K-A-C-E-S-R-C-T-K	-1.59696	-1.29049	4.578547	30.83419	-1.40282	-1.63588	27.68044
8	K-Q-K-A-C-E-S-R-C-T-K-L-E-Y-D	-0.6145	-1.04544	4.210649	7.300528	-1.78057	-1.59691	6.014428
9	C-E-S-R-C-T-K-L-E-Y-D-P-R-C-V	1.217389	-0.81995	8.846369	2.930048	-1.40647	-1.18977	6.974979
10	C-T-K-L-E-Y-D-P-R-C-V-Y-D-P-R	-0.43066	-1.26932	15.71256	3.578001	-2.0053	-1.45402	1.970958
11	E-Y-D-P-R-C-V-Y-D-P-R-G-H-T-G	-1.67995	-0.90905	5.737663	2.477852	-1.32823	-0.85221	1.602457
12	R-C-V-Y-D-P-R-G-H-T-G-T-T-N-Q	-0.04374	-1.32199	6.651678	14.41312	-2.64247	-1.83133	5.322504
13	D-P-R-G-H-T-G-T-T-N-Q-R-S-P-P	5.233717	-0.86873	10.14217	31.00757	-1.51666	-1.07005	5.380502
14	H-T-G-T-T-N-Q-R-S-P-P-G-E-R-T	5.05205	-0.92656	13.80843	50.37398	-1.37656	-0.98748	13.61322
15	T-N-Q-R-S-P-P-G-E-R-T-R-G-R-Q	5.396772	-0.96323	23.84259	47.59736	-2.26482	-1.91054	15.21628
16	S-P-P-G-E-R-T-R-G-R-Q-P-G-D-Y	101.6422	-1.54412	138.9754	75.73122	-2.21237	-1.5714	34.37273
17	E-R-T-R-G-R-Q-P-G-D-Y-D-D-D-R	94.74203	-0.78214	156.2092	79.27746	-1.69849	-0.65665	35.48695
18	G-R-Q-P-G-D-Y-D-D-D-R-R-Q-P-R	4.39623	-1.13399	128.5906	38.32581	-2.8064	-1.78989	33.40966
19	G-D-Y-D-D-D-R-R-Q-P-R-R-E-E-G	-1.70135	-3.26375	10.27734	12.89541	-4.55987	-4.30123	12.14392
20	D-D-R-R-Q-P-R-R-E-E-G-G-R-W-G	-1.30447	-2.61614	5.510301	23.62639	-3.12526	-2.60206	31.50405
21	Q-P-R-R-E-E-G-G-R-W-G-P-A-G-P	-1.63822	-2.68659	4.044611	33.37429	-3.34322	-3.11166	32.74187
22	E-E-G-G-R-W-G-P-A-G-P-R-E-R-E	-0.71288	-2.13091	10.18315	20.60521	-2.31348	-2.38675	9.042681
23	R-W-G-P-A-G-P-R-E-R-E-R-E-E-D	0.279429	-1.69453	7.663261	14.33005	-1.79531	-1.67325	3.067443
24	A-G-P-R-E-R-E-R-E-E-D-W-R-Q-P	-1.69042	-1.97168	4.2548	10.70637	-3.57795	-3.28326	5.536934
25	E-R-E-R-E-E-D-W-R-Q-P-R-E-D-W	5.472416	-1.74171	24.31029	38.3183	-1.67231	-1.68774	34.68093
26	E-E-D-W-R-Q-P-R-E-D-W-R-R-P-S	104.7279	-2.18768	119.0229	55.6263	-2.29825	-2.45572	12.731
27	R-Q-P-R-E-D-W-R-R-P-S-H-Q-Q-P	101.061	-1.35483	121.8896	77.30616	-1.71439	-1.68078	20.01693
28	E-D-W-R-R-P-S-H-Q-Q-P-R-K-I-R	13.94241	-1.01535	32.19087	71.18853	-0.04886	-1.36856	35.38292
29	R-P-S-H-Q-Q-P-R-K-I-R-P-E-G-R	5.028631	-1.58021	72.42044	77.746	-1.29973	-1.65364	35.61126
30	Q-Q-P-R-K-I-R-P-E-G-R-E-G-E-Q	3.2592	-0.924	143.969	67.49117	-1.47808	-1.6132	36.21804
31	K-I-R-P-E-G-R-E-G-E-Q-E-W-G-T	-1.39417	-1.12085	11.92243	13.38672	-1.99567	-2.01372	35.45769
32	E-G-R-E-G-E-Q-E-W-G-T-P-G-S-H	0.423326	-0.79234	12.94582	50.46335	-1.40024	-0.98384	28.57079
33	G-E-Q-E-W-G-T-P-G-S-H-V-R-E-E	2.268558	-1.39609	19.65561	47.14635	-2.10994	-1.5474	15.32433
34	W-G-T-P-G-S-H-V-R-E-E-T-S-R-N	1.668563	-1.06294	14.38033	31.02177	-1.47751	-1.16937	14.78174
35	G-S-H-V-R-E-E-T-S-R-N-N-P-F-Y	1.904169	-0.47352	12.29089	13.00512	-0.78828	-0.31569	6.951695
36	R-E-E-T-S-R-N-N-P-F-Y-F-P-S-R	0.664725	-1.01146	15.3664	37.84777	-2.14992	-1.574	20.1949
37	S-R-N-N-P-F-Y-F-P-S-R-R-F-S-T	2.879661	-3.86148	10.53053	25.83632	-3.82639	-4.72186	6.020433

38	P-F-Y-F-P-S-R-R-F-S-T-R-Y-G-N	-2.91062	-4.77006	12.3355	9.294225	-5.42918	-5.68308	2.134125
39	P-S-R-R-F-S-T-R-Y-G-N-Q-N-G-R	2.06314	-1.88039	10.47544	22.99433	-2.29209	-2.45767	11.51753
40	F-S-T-R-Y-G-N-Q-N-G-R-I-R-V-L	6.12271	-4.46206	12.66561	28.57142	-6.01261	-6.00344	11.45757
41	Y-G-N-Q-N-G-R-I-R-V-L-Q-R-F-D	6.982778	-4.67576	15.49696	27.14248	-4.67084	-4.99741	4.887234
42	N-G-R-I-R-V-L-Q-R-F-D-Q-R-S-R	-1.429	-2.08553	12.62039	13.28132	-1.57377	-2.43191	6.710459
43	R-V-L-Q-R-F-D-Q-R-S-R-Q-F-Q-N	0.558192	-2.76385	3.506153	7.233424	-3.43636	-3.68997	2.250496
44	R-F-D-Q-R-S-R-Q-F-Q-N-L-Q-N-H	1.333604	-3.00143	6.608565	24.15817	-3.00646	-3.21478	11.20161
45	R-S-R-Q-F-Q-N-L-Q-N-H-R-I-V-Q	-0.19648	-3.6533	9.781506	5.598545	-3.90371	-4.48453	4.733501
46	F-Q-N-L-Q-N-H-R-I-V-Q-I-E-A-K	-2.75446	-2.65648	3.76666	-2.57496	-4.54842	-4.58893	-2.43703
47	Q-N-H-R-I-V-Q-I-E-A-K-P-N-T-L	-0.00045	-1.76773	3.098764	2.529633	-1.5295	-2.06496	-0.46991
48	I-V-Q-I-E-A-K-P-N-T-L-V-L-P-K	2.034737	-1.03758	8.143733	8.81598	-2.60707	-2.67854	8.303972
49	E-A-K-P-N-T-L-V-L-P-K-H-A-D-A	10.7752	-1.93522	44.02096	55.05688	-2.42544	-2.46962	24.95336
50	N-T-L-V-L-P-K-H-A-D-A-D-N-I-L	12.15944	-1.21048	11.46783	5.907945	-1.5587	-2.06046	7.32343
51	L-P-K-H-A-D-A-D-N-I-L-V-I-Q-Q	8.132508	-1.47981	14.64615	18.9476	-2.65497	-2.86101	11.98281
52	A-D-A-D-N-I-L-V-I-Q-Q-G-Q-A-T	0.384171	-1.50346	12.15213	20.1544	-3.68035	-3.92887	13.79111
53	N-I-L-V-I-Q-Q-G-Q-A-T-V-T-V-A	1.146884	-1.58	19.91124	44.68331	-1.94136	-2.24317	23.43433
54	I-Q-Q-G-Q-A-T-V-T-V-A-N-G-N-N	0.646232	-0.94669	12.89486	28.10026	-2.63433	-2.48739	25.28107
55	Q-A-T-V-T-V-A-N-G-N-N-R-K-S-F	-0.16656	-0.53616	26.61677	49.27365	-4.83482	-4.99781	24.33136
56	T-V-A-N-G-N-N-R-K-S-F-N-L-D-E	5.625827	-1.80621	34.79922	58.66269	-2.57394	-2.43726	24.25644
57	G-N-N-R-K-S-F-N-L-D-E-G-H-A-L	-0.93051	-2.00036	20.56977	28.62635	-4.86913	-4.70138	10.34225
58	K-S-F-N-L-D-E-G-H-A-L-R-I-P-S	0.761425	-0.43665	13.33631	18.27332	-1.38584	-1.30039	10.85536
59	L-D-E-G-H-A-L-R-I-P-S-G-F-I-S	-0.43787	-0.89598	7.611756	12.01	-0.25115	-0.92492	8.041904
60	H-A-L-R-I-P-S-G-F-I-S-Y-I-L-N	0.80846	-0.73437	7.771843	21.93277	-1.58461	-1.32308	11.7051
61	I-P-S-G-F-I-S-Y-I-L-N-R-H-D-N	-2.01283	-2.6487	4.323331	15.78383	-2.74889	-2.83217	4.163405
62	F-I-S-Y-I-L-N-R-H-D-N-Q-N-L-R	-1.05096	-3.94832	3.66708	44.18075	-3.96186	-4.61132	6.74679
63	I-L-N-R-H-D-N-Q-N-L-R-V-A-K-I	-2.03015	-2.86501	4.514404	48.54251	-4.01368	-3.76003	14.79915
64	H-D-N-Q-N-L-R-V-A-K-I-S-M-P-V	0.492965	-1.23841	6.410253	33.1103	-2.80141	-3.09216	12.40274
65	N-L-R-V-A-K-I-S-M-P-V-N-T-P-G	1.927388	-0.2128	6.415708	33.53159	-1.01998	-0.84067	10.85406
66	A-K-I-S-M-P-V-N-T-P-G-Q-F-E-D	-0.56915	-2.21226	3.383392	10.25419	-3.38119	-2.44618	8.161963
67	M-P-V-N-T-P-G-Q-F-E-D-F-F-P-A	-2.73412	-0.83921	3.414023	7.067644	-5.42976	-5.5943	3.51444
68	T-P-G-Q-F-E-D-F-F-P-A-S-S-R-D	0.003539	-2.97657	7.355956	19.83838	-4.51405	-3.93523	13.97264
69	F-E-D-F-F-P-A-S-S-R-D-Q-S-S-Y	-2.56025	-3.61569	1.103913	-1.23681	-8.14269	-7.09461	-0.55163
70	F-P-A-S-S-R-D-Q-S-S-Y-L-Q-G-F	0.273181	-1.86596	3.170334	2.11726	-3.59848	-3.87715	6.816776
71	S-R-D-Q-S-S-Y-L-Q-G-F-S-R-N-T	0.483109	-1.8368	4.734895	1.604163	-1.272	-1.89775	7.356824
72	S-S-Y-L-Q-G-F-S-R-N-T-L-E-A-A	13.79686	-2.54002	33.5449	53.80098	-3.62127	-3.9157	23.86414
73	Q-G-F-S-R-N-T-L-E-A-A-F-N-A-E	6.05654	-1.66962	9.89048	32.18801	-1.84125	-2.42823	18.38054
74	R-N-T-L-E-A-A-F-N-A-E-F-N-E-I	1.693399	-1.26993	7.10495	14.35592	-1.19494	-2.16781	11.12055
75	E-A-A-F-N-A-E-F-N-E-I-R-R-V-L	-0.18233	-3.84404	8.502414	30.03316	-4.18012	-4.99315	16.58696
76	N-A-E-F-N-E-I-R-R-V-L-L-E-E-N	-1.57072	-3.36625	3.041913	6.82938	-3.60753	-3.96153	9.596066
77	N-E-I-R-R-V-L-L-E-E-N-A-G-G-E	1.18188	-0.72909	7.626726	28.32934	-1.16322	-1.70779	10.59939
78	R-V-L-L-E-E-N-A-G-G-E-Q-E-E-R	0.525892	-0.19888	9.526839	7.32294	-1.86918	-2.10006	9.251623
79	E-E-N-A-G-G-E-Q-E-E-R-G-Q-R-R	-0.63483	-2.14629	12.10787	18.16466	-2.78963	-3.32912	9.912691
80	G-G-E-Q-E-E-R-G-Q-R-R-W-S-T-R	-0.89763	-1.65671	3.746807	14.21608	-1.81044	-1.79885	7.126158
81	E-E-R-G-Q-R-R-W-S-T-R-S-S-E-N	-1.10001	-0.94261	7.70673	21.05212	-1.95703	-2.14538	10.27823
82	Q-R-R-W-S-T-R-S-S-E-N-N-E-G-V	-0.47445	-0.87996	13.98661	24.92618	-1.0377	-1.30231	9.42649

83	S-T-R-S-S-E-N-N-E-G-V-I-V-K-V	2.255613	-0.81844	10.58789	20.18157	-0.09821	-0.75039	9.731262
84	S-E-N-N-E-G-V-I-V-K-V-S-K-E-H	0.565426	-0.73057	5.916941	46.82522	-0.44573	-0.65583	14.34043
85	E-G-V-I-V-K-V-S-K-E-H-V-E-E-L	0.884226	-0.91452	3.777985	45.11822	-1.40306	-1.27132	5.23246
86	V-K-V-S-K-E-H-V-E-E-L-T-K-H-A	3.125025	-1.12302	2.574044	81.98156	-1.29007	-1.05787	8.149425
87	K-E-H-V-E-E-L-T-K-H-A-K-S-V-S	1.175013	-0.53455	3.898684	55.2159	-0.76822	-0.43064	11.73383
88	E-E-L-T-K-H-A-K-S-V-S-K-K-G-S	3.28455	-2.1558	6.100641	50.40856	-3.18763	-3.32008	13.61296
89	K-H-A-K-S-V-S-K-K-G-S-E-E-E-G	2.144801	-0.50534	4.945178	32.94093	-1.44733	-1.26846	9.146233
90	S-V-S-K-K-G-S-E-E-E-G-D-I-T-N	2.688383	-1.00897	4.365974	7.171135	-2.14077	-1.07131	6.719895
91	K-G-S-E-E-E-G-D-I-T-N-P-I-N-L	0.491442	-4.80469	7.024659	74.00956	-6.05139	-5.6899	1.529855
92	E-E-G-D-I-T-N-P-I-N-L-R-E-G-E	-0.42248	-3.32133	2.326791	76.37315	-3.29246	-3.37135	1.949524
93	I-T-N-P-I-N-L-R-E-G-E-P-D-L-S	0.628915	-2.37774	3.768603	26.45127	-2.40402	-2.71449	4.619271
94	I-N-L-R-E-G-E-P-D-L-S-N-N-F-G	-2.65711	-4.29953	2.007046	0.177289	-6.36404	-6.26035	-0.19891
95	E-G-E-P-D-L-S-N-N-F-G-K-L-F-E	0.56958	-1.66373	3.039817	10.21795	-0.70784	-1.67639	12.80745
96	D-L-S-N-N-F-G-K-L-F-E-V-K-P-D	0.032812	-2.36724	6.813636	25.79338	-2.5476	-2.90486	27.23237
97	N-F-G-K-L-F-E-V-K-P-D-K-K-N-P	1.609543	-1.20808	12.22713	77.75274	-0.55716	-1.53234	35.79457
98	L-F-E-V-K-P-D-K-K-N-P-Q-L-Q-D	0.765632	-0.99479	6.988228	46.77289	-0.61071	-1.45534	25.73757
99	K-P-D-K-K-N-P-Q-L-Q-D-L-D-M-M	-0.15602	-1.24799	3.475558	42.35868	-1.16042	-1.55434	16.10788
100	K-N-P-Q-L-Q-D-L-D-M-M-L-T-C-V	-1.15822	-1.23646	3.834192	39.20188	-0.91974	-1.54106	19.10049
101	L-Q-D-L-D-M-M-L-T-C-V-E-I-K-E	0.40628	-0.94894	2.228946	17.13975	-0.89563	-1.45728	10.31285
102	D-M-M-L-T-C-V-E-I-K-E-G-A-L-M	1.584321	-0.97945	9.13937	32.26898	-0.3729	-0.94917	17.72444
103	T-C-V-E-I-K-E-G-A-L-M-L-P-H-F	0.051058	-0.87718	9.682749	24.31602	-0.27534	-0.7372	21.23833
104	I-K-E-G-A-L-M-L-P-H-F-N-S-K-A	0.37463	-0.63113	11.92178	25.77038	-1.02924	-0.8116	13.20649
105	A-L-M-L-P-H-F-N-S-K-A-M-V-I-V	3.693317	-1.08289	21.74644	53.20744	-0.763	-0.74342	19.65838
106	P-H-F-N-S-K-A-M-V-I-V-V-V-N-K	-0.19588	-0.64352	4.091794	15.73264	-0.19166	-0.43107	4.960995
107	S-K-A-M-V-I-V-V-V-N-K-G-T-G-N	8.948462	-0.74749	11.82183	32.8327	-0.00316	-0.66073	16.42641
108	V-I-V-V-V-N-K-G-T-G-N-L-E-L-V	2.815637	-0.45061	4.906719	23.7942	-0.33195	-0.28351	8.258819
109	V-N-K-G-T-G-N-L-E-L-V-A-V-R-K	1.821475	-0.58334	5.060639	49.27519	-0.93315	-0.6153	11.52114
110	T-G-N-L-E-L-V-A-V-R-K-E-Q-Q-Q	5.755026	-0.88687	3.964197	64.76099	-1.52764	-0.89039	7.946722
111	E-L-V-A-V-R-K-E-Q-Q-Q-R-G-R-R	2.779256	-1.00459	4.556745	41.7388	-1.20761	-1.70708	13.79276
112	V-R-K-E-Q-Q-Q-R-G-R-R-E-E-E-E	-0.7306	-1.98496	3.032085	10.82474	-2.44588	-2.40121	5.486927
113	Q-Q-Q-R-G-R-R-E-E-E-E-D-E-D-E	0.555561	-0.48839	4.599313	8.327343	-1.52843	-1.18192	6.675725
114	G-R-R-E-E-E-E-D-E-D-E-E-E-E-G	1.050436	-1.03798	3.225533	4.970776	-2.36082	-1.48068	6.291722
115	E-E-E-D-E-D-E-E-E-E-G-S-N-R-E	-0.45226	-2.9844	-0.23085	13.62784	-4.68556	-4.2184	-2.52201
116	E-D-E-E-E-E-G-S-N-R-E-V-R-R-Y	-1.27293	-4.5817	1.620135	11.40208	-5.13086	-4.66275	6.718442
117	E-E-G-S-N-R-E-V-R-R-Y-T-A-R-L	3.225142	-2.48539	6.775563	20.7375	-4.11099	-4.56352	9.505198
118	N-R-E-V-R-R-Y-T-A-R-L-K-E-G-D	3.510736	-4.35489	9.855619	21.48321	-6.15202	-6.24847	4.563558
119	R-R-Y-T-A-R-L-K-E-G-D-V-F-I-M	0.324324	-3.75013	3.189184	-0.57566	-4.29187	-4.1765	0.816455
120	A-R-L-K-E-G-D-V-F-I-M-P-A-A-H	-1.43321	-1.59552	2.133976	3.466246	-1.76	-1.77813	3.551298
121	E-G-D-V-F-I-M-P-A-A-H-P-V-A-I	-0.05351	-1.16201	5.430812	22.62657	-1.06	-1.72718	17.24154
122	F-I-M-P-A-A-H-P-V-A-I-N-A-S-S	5.311083	-1.01477	5.096779	63.83618	-0.65664	-1.05178	21.005
123	A-A-H-P-V-A-I-N-A-S-S-E-L-H-L	3.994565	-0.93738	3.8405	57.62347	-0.62674	-0.56993	19.36232
124	V-A-I-N-A-S-S-E-L-H-L-L-G-F-G	0.534335	-1.19033	7.733454	25.0588	-1.40539	-0.9957	14.76579
125	A-S-S-E-L-H-L-L-G-F-G-I-N-A-E	0.233382	-1.36833	4.427056	18.77328	-1.74252	-2.08845	17.93215
126	L-H-L-L-G-F-G-I-N-A-E-N-N-H-R	-0.22166	-1.31261	3.604658	7.857842	-0.48333	-1.49514	13.12782
127	G-F-G-I-N-A-E-N-N-H-R-I-F-L-A	4.420579	-0.97226	5.452588	15.31423	-1.13986	-1.38603	13.30913

128	N-A-E-N-N-H-R-I-F-L-A-G-D-K-D	4.385259	-1.13582	23.78885	64.98063	-0.85484	-1.23699	26.38865
129	N-H-R-I-F-L-A-G-D-K-D-N-V-I-D	2.498449	-1.54271	19.18897	32.11885	-1.83682	-1.90744	11.06618
130	F-L-A-G-D-K-D-N-V-I-D-Q-I-E-K	0.385411	-1.60956	8.70718	29.24332	-2.4132	-2.14689	11.66448
131	D-K-D-N-V-I-D-Q-I-E-K-Q-A-K-D	2.048915	-1.15672	5.30862	20.75059	-1.45906	-1.1998	8.205495
132	V-I-D-Q-I-E-K-Q-A-K-D-L-A-F-P	0.116513	-0.21955	2.449824	17.38991	-0.25684	-0.31871	12.18103
133	I-E-K-Q-A-K-D-L-A-F-P-G-S-G-E	1.688853	-0.52652	6.992434	39.47771	-0.62198	-0.36719	24.75003
134	A-K-D-L-A-F-P-G-S-G-E-Q-V-E-K	4.887313	-0.17504	4.629524	63.67664	-0.65469	-0.32236	35.01905
135	A-F-P-G-S-G-E-Q-V-E-K-L-I-K-N	3.416727	0.543476	4.537165	36.67111	-1.80814	-1.28883	15.65822
136	S-G-E-Q-V-E-K-L-I-K-N-Q-K-E-S	1.690728	-0.80209	4.977953	46.95641	-1.18723	-1.1018	13.63121
137	V-E-K-L-I-K-N-Q-K-E-S-H-F-V-S	0.292926	-1.20195	3.360106	20.01658	-1.63476	-1.39449	9.531064
138	I-K-N-Q-K-E-S-H-F-V-S-A-R-P-Q	0.627639	-0.99542	5.973301	33.24773	-2.36784	-1.30785	10.80822
139	K-E-S-H-F-V-S-A-R-P-Q-S-Q-S-Q	-1.19089	-2.22065	0.752184	14.13455	-3.3484	-3.08824	9.134007
140	F-V-S-A-R-P-Q-S-Q-S-Q-S-P-S-S	0.743476	-2.27155	3.920178	12.2823	-3.59278	-3.11468	5.145278
141	R-P-Q-S-Q-S-Q-S-P-S-S-P-E-K-E	-1.61278	-5.61425	5.185441	-3.20812	-9.15315	-8.08324	-1.83727
142	Q-S-Q-S-P-S-S-P-E-K-E-S-P-E-K	-1.10026	-2.35776	1.590671	16.48993	-3.90891	-3.96924	3.160473
143	P-S-S-P-E-K-E-S-P-E-K-E-D-Q-E	-0.98098	-1.81189	0.530596	2.398958	-2.78423	-2.37454	-0.11548
144	E-K-E-S-P-E-K-E-D-Q-E-E-E-N-Q	-1.9921	-0.63426	-0.31813	1.260656	-1.92195	-1.93519	0.402007
145	P-E-K-E-D-Q-E-E-E-N-Q-G-G-K-G	-0.62386	-1.30003	1.226884	8.736883	-1.25358	-1.54171	12.43007
146	D-Q-E-E-E-N-Q-G-G-K-G-P-L-L-S	-0.30523	-1.6141	5.276036	22.68592	-1.86188	-2.1375	16.86427
147	E-N-Q-G-G-K-G-P-L-L-S-I-L-K-A	1.091935	-2.28209	6.756705	46.14077	-2.84575	-2.61573	16.91618
148	Q-G-G-K-G-P-L-L-S-I-L-K-A-F-N	0.050847	-1.14198	2.290236	39.98288	-2.13063	-1.81654	15.93818
	m (blanks)	13064.72	11944.52	11980.02	11826.06	30213.72	12411.71	13647.98
	s (blanks)	405.2419	496.9434	279.4139	579.7481	12783.8	440.7967	1229.712

Table A4: Calculated Z-scores of Ara h 1 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 1 for each peanut-allergic patient (patients 22-23) and tolerant patient (24-28) are listed. Identified candidate diagnostic peptides are highlighted in light blue.

	Patient No. →	22	23	24	25	26	27	28
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	K-S-S-P-Y-Q-K-K-T-E-N-P-C-A-Q	-0.96593	-1.88741	-1.9334	-5.48229	-1.16104	-1.24353	-1.89544
2	Y-Q-K-K-T-E-N-P-C-A-Q-R-C-L-Q	-0.22367	-1.29926	-1.76534	-5.1789	-0.75234	-1.18467	-1.54099
3	T-E-N-P-C-A-Q-R-C-L-Q-S-C-Q-Q	-1.1109	-0.63789	-1.86761	-5.43652	-1.19922	-1.23916	-1.7233
4	C-A-Q-R-C-L-Q-S-C-Q-Q-E-P-D-D	-0.9305	4.028964	-1.65366	-4.28096	-0.55941	-0.5893	-1.30832
5	C-L-Q-S-C-Q-Q-E-P-D-D-L-K-Q-K	-0.94482	73.39521	-1.95855	-4.67359	-1.09063	-0.73853	-1.78404
6	C-Q-Q-E-P-D-D-L-K-Q-K-A-C-E-S	-2.01721	105.5123	-2.3351	-4.37118	-1.06265	-1.3399	-1.67991
7	P-D-D-L-K-Q-K-A-C-E-S-R-C-T-K	-1.76552	3.469808	-1.70308	-3.8795	-0.76011	-0.67009	-1.2161
8	K-Q-K-A-C-E-S-R-C-T-K-L-E-Y-D	-1.36655	-0.96888	-1.96856	-4.84412	-1.24488	-0.12082	-1.13246
9	C-E-S-R-C-T-K-L-E-Y-D-P-R-C-V	-0.6769	-1.90235	-1.41533	-4.81263	-0.86405	-0.41627	-0.73189
10	C-T-K-L-E-Y-D-P-R-C-V-Y-D-P-R	-1.36556	-2.6392	-1.99698	-5.17975	-1.18146	-0.47228	-1.16717
11	E-Y-D-P-R-C-V-Y-D-P-R-G-H-T-G	-1.31096	-1.63034	-1.3407	-3.39335	-0.2387	0.039489	-0.43825
12	R-C-V-Y-D-P-R-G-H-T-G-T-T-N-Q	-1.25763	-1.1982	-2.04591	-6.10252	-1.8111	-0.42561	-1.28305
13	D-P-R-G-H-T-G-T-T-N-Q-R-S-P-P	-0.47983	0.156837	-1.57647	-4.30831	-0.67615	0.417156	-0.50098
14	H-T-G-T-T-N-Q-R-S-P-P-G-E-R-T	-0.66363	2.51993	-1.65629	-3.93559	-1.1396	0.609136	-0.25981
15	T-N-Q-R-S-P-P-G-E-R-T-R-G-R-Q	-1.10583	0.800835	-2.08916	-5.66977	-2.26942	0.244989	-1.03663

16	S-P-P-G-E-R-T-R-G-R-Q-P-G-D-Y	-0.71889	68.87155	-1.84813	-6.58955	-2.13665	-0.05873	-0.53409
17	E-R-T-R-G-R-Q-P-G-D-Y-D-D-D-R	2.213756	76.82545	-1.31895	-6.90829	-1.20418	0.849557	0.241959
18	G-R-Q-P-G-D-Y-D-D-D-R-R-Q-P-R	-1.1273	8.727532	-2.01196	-7.3864	-2.28928	0.138119	-0.66491
19	G-D-Y-D-D-D-R-R-Q-P-R-R-E-E-G	-2.74601	-4.90397	-3.46436	-7.90928	-4.20499	-3.72737	-4.28444
20	D-D-R-R-Q-P-R-R-E-E-G-G-R-W-G	-2.43837	-3.4936	-2.25873	-8.00302	-2.64256	-2.30754	-2.70192
21	Q-P-R-R-E-E-G-G-R-W-G-P-A-G-P	-2.96305	-3.62384	-2.88056	-8.06445	-2.69409	-2.91109	-2.73536
22	E-E-G-G-R-W-G-P-A-G-P-R-E-R-E	-1.61768	-2.82585	-2.3444	-6.5753	-1.92236	-2.05578	-2.42958
23	R-W-G-P-A-G-P-R-E-R-E-R-E-E-D	-0.88741	-0.67394	-1.99686	-5.67864	-1.07129	-1.52214	-1.70827
24	A-G-P-R-E-R-E-R-E-E-D-W-R-Q-P	-1.96477	-2.47122	-3.2808	-7.34573	-2.73166	-2.02215	-2.87608
25	E-R-E-R-E-E-D-W-R-Q-P-R-E-D-W	1.587921	4.444458	-1.63233	-5.29603	-1.37911	-1.44882	-1.60814
26	E-E-D-W-R-Q-P-R-E-D-W-R-R-P-S	-1.82889	-0.42065	-2.10399	-5.62975	-1.57063	-1.78652	-1.89796
27	R-Q-P-R-E-D-W-R-R-P-S-H-Q-Q-P	-0.57374	1.736924	-1.88224	-4.98528	-0.92097	-1.21297	-1.54323
28	E-D-W-R-R-P-S-H-Q-Q-P-R-K-I-R	-0.66931	99.26503	-1.44464	-2.9662	-0.31777	-0.31715	-1.20496
29	R-P-S-H-Q-Q-P-R-K-I-R-P-E-G-R	-0.10115	103.67	-1.74402	-4.11382	-0.62705	-0.6785	-1.44666
30	Q-Q-P-R-K-I-R-P-E-G-R-E-G-E-Q	-0.64085	61.2642	-1.56681	-3.61118	-0.75149	-0.63052	-1.40217
31	K-I-R-P-E-G-R-E-G-E-Q-E-W-G-T	-0.94825	-0.36632	-2.31787	-5.20441	-1.64788	-1.16359	-1.69845
32	E-G-R-E-G-E-Q-E-W-G-T-P-G-S-H	-1.00753	1.050155	-1.26259	-5.39806	-0.7756	-0.23816	-0.60607
33	G-E-Q-E-W-G-T-P-G-S-H-V-R-E-E	-1.00291	2.974034	-1.58262	-5.89941	-1.15947	-0.37596	-1.20982
34	W-G-T-P-G-S-H-V-R-E-E-T-S-R-N	-0.42901	0.343593	-1.55681	-4.93593	-0.73769	-0.19858	-0.67666
35	G-S-H-V-R-E-E-T-S-R-N-N-P-F-Y	-0.51856	-0.56572	-0.71089	-4.22168	0.179986	0.6312	0.010161
36	R-E-E-T-S-R-N-N-P-F-Y-F-P-S-R	0.130909	0.802006	-1.74933	-5.85427	-1.22835	-0.25625	-1.0514
37	S-R-N-N-P-F-Y-F-P-S-R-R-F-S-T	-1.37067	0.952129	-4.91258	-5.3611	-4.21586	-1.87428	-4.11434
38	P-F-Y-F-P-S-R-R-F-S-T-R-Y-G-N	-2.84609	-3.60192	-6.14402	-7.83549	-5.97018	-2.69523	-4.94688
39	P-S-R-R-F-S-T-R-Y-G-N-Q-N-G-R	-1.48485	-2.36242	-3.10558	-5.86693	-2.89298	-0.75458	-1.35295
40	F-S-T-R-Y-G-N-Q-N-G-R-I-R-V-L	-3.17611	-3.98037	-6.35998	-10.7655	-7.11584	-2.46929	-4.90415
41	Y-G-N-Q-N-G-R-I-R-V-L-Q-R-F-D	-3.62562	-3.40967	-5.22683	-10.0711	-5.43696	-2.39852	-3.83078
42	N-G-R-I-R-V-L-Q-R-F-D-Q-R-S-R	-2.20673	-1.4678	-2.84051	-7.3006	-2.8337	-0.72208	-0.81155
43	R-V-L-Q-R-F-D-Q-R-S-R-Q-F-Q-N	-3.25074	-3.78535	-3.17762	-8.48035	-3.83661	-3.06263	-3.49398
44	R-F-D-Q-R-S-R-Q-F-Q-N-L-Q-N-H	-2.77069	-3.70588	-2.73329	-6.78921	-3.27117	-2.97671	-3.14436
45	R-S-R-Q-F-Q-N-L-Q-N-H-R-I-V-Q	-2.95689	-5.37591	-4.27938	-7.89753	-3.90011	-3.88248	-4.46512
46	F-Q-N-L-Q-N-H-R-I-V-Q-I-E-A-K	-2.88042	-6.06893	-4.93681	-7.77337	-4.53564	-4.27985	-4.66242
47	Q-N-H-R-I-V-Q-I-E-A-K-P-N-T-L	-1.77684	-1.81969	-2.07833	-4.39299	-1.64245	-1.60165	-1.95572
48	I-V-Q-I-E-A-K-P-N-T-L-V-L-P-K	-0.2137	0.157028	-2.91585	-6.1498	-2.86155	-2.09678	-2.52997
49	E-A-K-P-N-T-L-V-L-P-K-H-A-D-A	0.80656	8.397982	-2.4134	-5.41115	-2.39937	-1.51247	-2.43391
50	N-T-L-V-L-P-K-H-A-D-A-D-N-I-L	-1.01459	0.016104	-1.83741	-4.96782	-1.80415	-1.44871	-1.81019
51	L-P-K-H-A-D-A-D-N-I-L-V-I-Q-Q	1.32399	2.546529	-2.74966	-5.65179	-2.92503	-0.97919	-2.86152
52	A-D-A-D-N-I-L-V-I-Q-Q-G-Q-A-T	-1.60849	6.666821	-3.9495	-6.02428	-3.63972	-2.53526	-3.88086
53	N-I-L-V-I-Q-Q-G-Q-A-T-V-T-V-A	-0.63796	8.94339	-2.17572	-4.63878	-1.83868	-0.94452	-2.03524
54	I-Q-Q-G-Q-A-T-V-T-V-A-N-G-N-N	-0.21328	2.742649	-2.63951	-4.8146	-2.77781	-0.72892	-2.38194
55	Q-A-T-V-T-V-A-N-G-N-N-R-K-S-F	-2.02488	-2.74472	-5.07179	-7.42406	-5.23848	-2.90401	-4.51457
56	T-V-A-N-G-N-N-R-K-S-F-N-L-D-E	-0.39295	1.547072	-2.2155	-6.3912	-2.79791	-0.61471	-1.84534
57	G-N-N-R-K-S-F-N-L-D-E-G-H-A-L	-2.05016	-3.50132	-4.7703	-8.22817	-5.14956	-2.39285	-4.38543
58	K-S-F-N-L-D-E-G-H-A-L-R-I-P-S	-0.25904	-1.79172	-1.7461	-5.27804	-1.38826	-0.26538	-0.91124
59	L-D-E-G-H-A-L-R-I-P-S-G-F-I-S	-0.87128	-0.76361	-1.28544	-3.05431	-0.46119	0.026135	-0.37126
60	H-A-L-R-I-P-S-G-F-I-S-Y-I-L-N	0.045053	-0.72693	-1.17563	-6.00307	-1.81202	0.050173	-0.68295

61	I-P-S-G-F-I-S-Y-I-L-N-R-H-D-N	-2.15687	-3.28503	-2.81353	-6.62933	-3.01534	-1.04473	-1.8101
62	F-I-S-Y-I-L-N-R-H-D-N-Q-N-L-R	-2.90558	-3.84163	-4.74124	-7.4773	-4.96383	-2.56415	-3.71703
63	I-L-N-R-H-D-N-Q-N-L-R-V-A-K-I	-1.03532	-3.44675	-4.38729	-8.68597	-4.51924	-1.98556	-2.84062
64	H-D-N-Q-N-L-R-V-A-K-I-S-M-P-V	-0.88733	-1.90299	-2.87013	-8.60814	-3.8618	-0.8994	-1.98835
65	N-L-R-V-A-K-I-S-M-P-V-N-T-P-G	-0.64355	-1.32081	-1.40693	-7.39942	-1.74408	0.555563	-0.16868
66	A-K-I-S-M-P-V-N-T-P-G-Q-F-E-D	-2.35598	-3.34461	-3.46685	-9.69208	-3.98484	0.008007	-1.66147
67	M-P-V-N-T-P-G-Q-F-E-D-F-F-P-A	-2.79829	-6.1347	-5.02035	-8.69216	-6.38315	-3.08785	-5.28328
68	T-P-G-Q-F-E-D-F-F-P-A-S-S-R-D	-3.57138	-4.77384	-4.48884	-8.66395	-5.05552	-3.31468	-4.62915
69	F-E-D-F-F-P-A-S-S-R-D-Q-S-S-Y	-3.94157	-8.96239	-8.01268	-12.0841	-8.64472	-5.90745	-8.16398
70	F-P-A-S-S-R-D-Q-S-S-Y-L-Q-G-F	-2.01852	-3.86933	-3.94799	-7.31701	-4.12451	-2.9561	-4.0892
71	S-R-D-Q-S-S-Y-L-Q-G-F-S-R-N-T	-1.06273	0.333018	-2.12839	-4.14836	-2.20852	-1.52996	-2.15398
72	S-S-Y-L-Q-G-F-S-R-N-T-L-E-A-A	3.597006	39.95089	-4.20983	-6.26647	-4.17238	-2.59155	-4.02274
73	Q-G-F-S-R-N-T-L-E-A-A-F-N-A-E	-0.52198	47.08792	-2.10013	-4.85823	-2.11863	-1.54834	-2.14014
74	R-N-T-L-E-A-A-F-N-A-E-F-N-E-I	0.069256	16.20381	-1.80885	-6.77821	-2.079	-1.01855	-1.73
75	E-A-A-F-N-A-E-F-N-E-I-R-R-V-L	-1.78469	9.029146	-4.88754	-6.46461	-5.26286	-3.79904	-4.76696
76	N-A-E-F-N-E-I-R-R-V-L-L-E-E-N	-2.31255	0.660755	-4.40296	-6.54036	-4.19647	-2.85777	-3.84284
77	N-E-I-R-R-V-L-L-E-E-N-A-G-G-E	0.069005	3.842076	-1.81044	-3.23003	-1.40076	-0.5564	-1.43547
78	R-V-L-L-E-E-N-A-G-G-E-Q-E-E-R	-0.07618	-0.13095	-2.25377	-4.15397	-2.71525	-1.07257	-2.15288
79	E-E-N-A-G-G-E-Q-E-E-R-G-Q-R-R	-1.4575	-2.31006	-3.52853	-5.12545	-3.34151	-2.09516	-2.89655
80	G-G-E-Q-E-E-R-G-Q-R-R-W-S-T-R	-1.01058	-1.70582	-1.76681	-5.56822	-2.20975	-0.73135	-1.30343
81	E-E-R-G-Q-R-R-W-S-T-R-S-S-E-N	-0.87961	-1.95928	-2.17875	-4.59396	-2.49261	-0.92536	-1.8309
82	Q-R-R-W-S-T-R-S-S-E-N-N-E-G-V	-0.12028	-1.67085	-1.74452	-4.28946	-1.70393	-0.15521	-0.91505
83	S-T-R-S-S-E-N-N-E-G-V-I-V-K-V	-0.68781	0.031608	-0.93823	-2.55198	-0.20397	0.471262	-0.0969
84	S-E-N-N-E-G-V-I-V-K-V-S-K-E-H	0.097479	0.417683	-0.82879	-3.60745	-0.6192	0.595277	-0.01427
85	E-G-V-I-V-K-V-S-K-E-H-V-E-E-L	-0.51303	-1.63809	-1.44765	-5.02007	-1.48957	0.166225	-0.52785
86	V-K-V-S-K-E-H-V-E-E-L-T-K-H-A	-0.59467	-0.99365	-1.44338	-5.13499	-1.37344	0.226367	-0.17213
87	K-E-H-V-E-E-L-T-K-H-A-K-S-V-S	0.16758	0.865034	-0.90147	-5.30604	-0.91029	0.852171	0.330606
88	E-E-L-T-K-H-A-K-S-V-S-K-K-G-S	-1.82794	-2.95619	-3.90883	-8.48398	-4.10911	1.347697	-2.4566
89	K-H-A-K-S-V-S-K-K-G-S-E-E-E-G	-1.2882	-1.55618	-1.63232	-8.18078	-1.98714	-0.01677	-0.32277
90	S-V-S-K-K-G-S-E-E-E-G-D-I-T-N	-1.44028	-0.01346	-1.49709	-8.54417	-2.47469	0.757111	-0.04766
91	K-G-S-E-E-E-G-D-I-T-N-P-I-N-L	-1.76289	-6.15943	-5.39685	-9.94773	-6.62282	-4.36613	-5.42499
92	E-E-G-D-I-T-N-P-I-N-L-R-E-G-E	-1.91574	-3.88243	-3.19721	-7.22899	-3.94755	-2.82767	-3.34837
93	I-T-N-P-I-N-L-R-E-G-E-P-D-L-S	-1.68446	-2.13366	-2.70067	-6.33934	-2.95605	-2.55337	-2.79313
94	I-N-L-R-E-G-E-P-D-L-S-N-N-F-G	-4.10653	-5.1647	-6.17546	-9.29722	-6.79894	-3.85684	-6.22619
95	E-G-E-P-D-L-S-N-N-F-G-K-L-F-E	-0.43904	10.14929	-1.68286	-3.17693	-1.535	-1.31479	-1.702
96	D-L-S-N-N-F-G-K-L-F-E-V-K-P-D	-1.09976	98.7807	-2.96299	-4.76067	-3.15548	-2.3293	-2.97098
97	N-F-G-K-L-F-E-V-K-P-D-K-K-N-P	-0.43547	109.2173	-1.23506	-2.86099	-1.12575	-0.84298	-1.16425
98	L-F-E-V-K-P-D-K-K-N-P-Q-L-Q-D	0.552438	93.36322	-1.18874	-3.94505	-1.44728	-0.81265	-1.19686
99	K-P-D-K-K-N-P-Q-L-Q-D-L-D-M-M	-0.60961	42.15726	-1.63249	-4.16139	-1.7959	-0.80002	-1.19815
100	K-N-P-Q-L-Q-D-L-D-M-M-L-T-C-V	-0.89098	11.42406	-1.44017	-3.32093	-2.01379	-0.59575	-1.26307
101	L-Q-D-L-D-M-M-L-T-C-V-E-I-K-E	-0.07294	3.784291	-1.26922	-3.28153	-1.54154	-0.53136	-0.74456
102	D-M-M-L-T-C-V-E-I-K-E-G-A-L-M	-0.51002	1.546282	-0.94538	-3.30079	-0.93388	-0.34005	-0.5694
103	T-C-V-E-I-K-E-G-A-L-M-L-P-H-F	-0.16756	-0.43497	-1.04727	-4.17986	-0.89873	-0.24061	-0.45506
104	I-K-E-G-A-L-M-L-P-H-F-N-S-K-A	-0.00764	-0.71608	-1.20391	-4.82348	-1.48337	0.513301	-0.42273
105	A-L-M-L-P-H-F-N-S-K-A-M-V-I-V	0.064219	1.607191	-1.19854	-3.07983	-1.08471	0.023127	-0.29375

106	P-H-F-N-S-K-A-M-V-I-V-V-V-N-K	-0.45085	-1.40921	-0.95869	-2.31421	-0.83743	0.247611	-0.37433
107	S-K-A-M-V-I-V-V-V-N-K-G-T-G-N	-0.19941	-0.46304	-0.97818	-3.04711	-0.65044	0.629788	-0.22597
108	V-I-V-V-V-N-K-G-T-G-N-L-E-L-V	-0.10304	0.046326	-0.73713	-3.02069	-0.54341	0.792302	0.171513
109	V-N-K-G-T-G-N-L-E-L-V-A-V-R-K	0.023356	-0.23746	-0.97552	-3.25464	-1.05292	0.443865	-0.09766
110	T-G-N-L-E-L-V-A-V-R-K-E-Q-Q-Q	-0.22143	0.846341	-1.22364	-5.16306	-1.38398	0.31351	0.070047
111	E-L-V-A-V-R-K-E-Q-Q-Q-R-G-R-R	-0.01233	1.70455	-2.00781	-6.60302	-2.22503	0.533122	-0.80269
112	V-R-K-E-Q-Q-Q-R-G-R-R-E-E-E-E	-1.43443	-2.02255	-2.5771	-8.28375	-3.2421	-0.75711	-1.44138
113	Q-Q-Q-R-G-R-R-E-E-E-E-D-E-D-E	-0.2868	-2.06331	-1.58545	-8.34046	-2.37237	0.265208	-0.33481
114	G-R-R-E-E-E-E-D-E-D-E-E-E-E-G	-1.36932	-1.50447	-1.66291	-8.83286	-2.81345	0.725189	-0.42721
115	E-E-E-D-E-D-E-E-E-E-G-S-N-R-E	-3.89408	-5.2525	-3.71621	-7.61443	-4.98856	-3.81668	-4.52105
116	E-D-E-E-E-E-G-S-N-R-E-V-R-R-Y	-1.19888	-5.26656	-4.57091	-7.95208	-5.55482	-4.16882	-4.61318
117	E-E-G-S-N-R-E-V-R-R-Y-T-A-R-L	-1.82031	-3.969	-4.57504	-7.61459	-5.21431	-3.56644	-4.36858
118	N-R-E-V-R-R-Y-T-A-R-L-K-E-G-D	-1.64969	-5.83612	-5.99515	-8.94103	-6.81823	-4.62704	-6.15757
119	R-R-Y-T-A-R-L-K-E-G-D-V-F-I-M	-3.30176	-2.94949	-4.24546	-4.74492	-4.61798	-3.73769	-4.34381
120	A-R-L-K-E-G-D-V-F-I-M-P-A-A-H	-1.24071	4.240484	-1.91398	5.975579	-2.07006	-1.4699	-1.83221
121	E-G-D-V-F-I-M-P-A-A-H-P-V-A-I	-0.46455	22.44662	-1.33396	-3.00896	-1.58091	-1.07645	-1.45141
122	F-I-M-P-A-A-H-P-V-A-I-N-A-S-S	-0.20312	1.916798	-1.11275	-3.8424	-0.99729	-0.1043	-0.43932
123	A-A-H-P-V-A-I-N-A-S-S-E-L-H-L	0.29908	0.881559	-0.71629	-3.56839	-0.60245	0.180781	-0.242
124	V-A-I-N-A-S-S-E-L-H-L-L-G-F-G	-0.78204	-0.80642	-1.46373	-4.05807	-1.47796	-0.2122	-0.61168
125	A-S-S-E-L-H-L-L-G-F-G-I-N-A-E	-0.28197	-0.68726	-2.1274	-1.78915	-2.28071	-1.14109	-1.65696
126	L-H-L-L-G-F-G-I-N-A-E-N-N-H-R	-0.72338	-0.71928	-1.78729	-1.7079	-1.4751	-0.85817	-1.33199
127	G-F-G-I-N-A-E-N-N-H-R-I-F-L-A	0.021241	-1.85202	-1.60154	-3.65235	-1.71196	-0.37891	-1.26089
128	N-A-E-N-N-H-R-I-F-L-A-G-D-K-D	0.597911	0.698823	-1.05483	-3.95982	-1.4164	0.041232	-0.55475
129	N-H-R-I-F-L-A-G-D-K-D-N-V-I-D	0.353081	-1.0316	-1.8242	-4.32249	-2.14159	-0.77481	-1.15323
130	F-L-A-G-D-K-D-N-V-I-D-Q-I-E-K	-0.68109	-1.89161	-2.71347	-4.91345	-2.4049	-1.38392	-1.78431
131	D-K-D-N-V-I-D-Q-I-E-K-Q-A-K-D	0.026234	-1.50698	-1.6048	-3.24169	-1.51328	-0.34256	-0.73462
132	V-I-D-Q-I-E-K-Q-A-K-D-L-A-F-P	-0.09504	0.0538	-0.53057	-1.50892	-0.47197	1.114052	0.341803
133	I-E-K-Q-A-K-D-L-A-F-P-G-S-G-E	-0.06977	1.582521	-0.51844	9.373499	-0.60908	0.575843	0.228411
134	A-K-D-L-A-F-P-G-S-G-E-Q-V-E-K	0.537195	28.85953	-0.62821	-0.59609	-1.00759	0.851699	0.456705
135	A-F-P-G-S-G-E-Q-V-E-K-L-I-K-N	-0.2464	4.745072	-1.89834	-6.72622	-2.48935	0.429462	-0.53325
136	S-G-E-Q-V-E-K-L-I-K-N-Q-K-E-S	-0.4134	-0.67259	-1.6125	-6.84798	-2.04693	0.298238	-0.53982
137	V-E-K-L-I-K-N-Q-K-E-S-H-F-V-S	-0.13159	-1.34983	-1.69171	-8.03865	-2.37266	0.39802	-0.38793
138	I-K-N-Q-K-E-S-H-F-V-S-A-R-P-Q	-0.31254	-0.83526	-1.51017	-8.75554	-2.51362	0.273687	0.008129
139	K-E-S-H-F-V-S-A-R-P-Q-S-Q-S-Q	-2.62867	-2.59702	-2.4771	-6.96425	-3.47161	-2.53391	-2.7745
140	F-V-S-A-R-P-Q-S-Q-S-Q-S-P-S-S	-2.36492	-3.30074	-2.8715	-7.24818	-3.83652	-2.69975	-3.11195
141	R-P-Q-S-Q-S-Q-S-P-S-S-P-E-K-E	-4.98134	-9.04071	-7.28264	-11.4012	-9.40041	-5.87239	-8.62151
142	Q-S-Q-S-P-S-S-P-E-K-E-S-P-E-K	-3.11686	-4.01253	-3.84125	-7.43564	-4.39026	-2.6927	-3.83887
143	P-S-S-P-E-K-E-S-P-E-K-E-D-Q-E	-2.45398	-2.09637	-2.58219	-5.40626	-3.05632	-2.3048	-2.59593
144	E-K-E-S-P-E-K-E-D-Q-E-E-E-N-Q	-1.68344	-0.7066	-2.10358	-3.94709	-2.29124	-1.49827	-2.04934
145	P-E-K-E-D-Q-E-E-E-N-Q-G-G-K-G	-1.50593	1.313218	-1.63151	-3.75374	-1.95518	-1.37046	-1.51578
146	D-Q-E-E-E-N-Q-G-G-K-G-P-L-L-S	-1.13567	3.263379	-1.74451	-4.74822	-2.42087	-1.43382	-1.89263
147	E-N-Q-G-G-K-G-P-L-L-S-I-L-K-A	-0.87581	0.331808	-3.01931	-5.80164	-3.8935	-2.14828	-2.74216
148	Q-G-G-K-G-P-L-L-S-I-L-K-A-F-N	-0.33289	-0.13049	-1.8276	-4.37701	-2.81068	-1.17849	-1.48254
	m (blanks)	13089.69	12093.18	13360.35	33476.14	26609.57	13484.05	12349.44
	s (blanks)	476.6536	439.4543	499.9719	2886.569	9751.287	375.3086	414.1513

Table A5: Calculated Z-scores of Ara h 1 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 1 for each peanut-tolerant patient (patients 29-35) are listed. Identified candidate diagnostic peptides are highlighted in light blue.

	Patient No. →	29	30	31	32	33	34	35
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	K-S-S-P-Y-Q-K-K-T-E-N-P-C-A-Q	-1.96098	-2.40195	-0.81397	-1.78074	-1.69102	-2.00107	-2.50144
2	Y-Q-K-K-T-E-N-P-C-A-Q-R-C-L-Q	-1.71713	-1.85872	-0.68889	-1.71758	-1.36501	-1.0472	-2.40786
3	T-E-N-P-C-A-Q-R-C-L-Q-S-C-Q-Q	-1.83232	-2.32718	-1.07615	-2.05178	-1.80273	-1.57224	-2.71814
4	C-A-Q-R-C-L-Q-S-C-Q-Q-E-P-D-D	-1.16849	-1.94313	-0.09479	-1.48779	-1.36059	-0.43869	-2.00044
5	C-L-Q-S-C-Q-Q-E-P-D-D-L-K-Q-K	-2.00628	-2.11968	-0.69619	-1.96348	-2.07297	-0.52157	-2.71761
6	C-Q-Q-E-P-D-D-L-K-Q-K-A-C-E-S	-1.95992	-2.29368	-0.88767	-2.12455	-2.12152	-1.2312	-2.75647
7	P-D-D-L-K-Q-K-A-C-E-S-R-C-T-K	-1.19909	-1.74503	-0.37792	-1.64453	-1.44126	0.390454	-1.99683
8	K-Q-K-A-C-E-S-R-C-T-K-L-E-Y-D	-1.36374	-1.55097	-0.39419	-1.65111	-1.78716	0.278091	-2.146
9	C-E-S-R-C-T-K-L-E-Y-D-P-R-C-V	-0.80363	-1.10953	0.455956	-1.24625	-1.1528	1.490519	-1.55207
10	C-T-K-L-E-Y-D-P-R-C-V-Y-D-P-R	-1.19466	-1.59953	-0.52083	-1.64188	-1.47003	0.782012	-2.23772
11	E-Y-D-P-R-C-V-Y-D-P-R-G-H-T-G	-0.43086	-0.77084	-0.04499	-0.82984	-0.98366	1.848603	-1.40551
12	R-C-V-Y-D-P-R-G-H-T-G-T-T-N-Q	-1.08522	-1.5556	-0.81931	-1.84505	-1.80461	0.613636	-2.50472
13	D-P-R-G-H-T-G-T-T-N-Q-R-S-P-P	-0.40748	-0.81841	0.43405	-1.10117	-0.52077	2.272838	-1.45777
14	H-T-G-T-T-N-Q-R-S-P-P-G-E-R-T	-0.16359	-0.53245	0.468962	-1.00479	-0.58585	2.471696	-1.28467
15	T-N-Q-R-S-P-P-G-E-R-T-R-G-R-Q	-1.09609	-1.34363	-0.25253	-1.78121	-1.51069	1.513614	-2.27749
16	S-P-P-G-E-R-T-R-G-R-Q-P-G-D-Y	-0.67311	-1.11558	0.125521	-1.37477	-1.25372	1.064519	-1.91362
17	E-R-T-R-G-R-Q-P-G-D-Y-D-D-D-R	0.192743	-0.0466	1.006891	-0.79795	-0.3607	1.836339	-0.99402
18	G-R-Q-P-G-D-Y-D-D-D-R-R-Q-P-R	-0.667	-0.9999	-0.00244	-1.67302	-1.39058	2.22806	-2.14727
19	G-D-Y-D-D-D-R-R-Q-P-R-R-E-E-G	-4.48229	-4.55056	-3.27029	-4.23338	-4.2206	-3.31441	-4.76474
20	D-D-R-R-Q-P-R-R-E-E-G-G-R-W-G	-2.82402	-3.155	-1.84359	-2.61513	-2.90935	-2.53401	-3.14969
21	Q-P-R-R-E-E-G-G-R-W-G-P-A-G-P	-3.12714	-3.46375	-2.10791	-2.90911	-3.09689	-3.1318	-3.20343
22	E-E-G-G-R-W-G-P-A-G-P-R-E-R-E	-2.58628	-2.88043	-1.57822	-2.34018	-2.46505	-2.17455	-2.85083
23	R-W-G-P-A-G-P-R-E-R-E-R-E-E-D	-1.83231	-2.25652	-0.96241	-1.66278	-1.6223	-2.45344	-2.425
24	A-G-P-R-E-R-E-R-E-E-D-W-R-Q-P	-3.27316	-3.81312	-2.15165	-3.2158	-3.14433	-3.64954	-3.78977
25	E-R-E-R-E-E-D-W-R-Q-P-R-E-D-W	-1.98915	-2.31693	-0.83361	-1.5388	-1.64667	-1.73639	-2.48978
26	E-E-D-W-R-Q-P-R-E-D-W-R-R-P-S	-1.98414	-2.69081	-1.26301	-1.94332	-2.05191	-1.6568	-2.61789
27	R-Q-P-R-E-D-W-R-R-P-S-H-Q-Q-P	-1.61314	-2.37918	-0.88226	-1.72193	-1.66359	-1.00035	-2.45924
28	E-D-W-R-R-P-S-H-Q-Q-P-R-K-I-R	-1.26404	-1.62897	-0.50543	-1.65135	-1.47052	2.772519	-1.93226
29	R-P-S-H-Q-Q-P-R-K-I-R-P-E-G-R	-1.24047	-1.84867	-0.76907	-1.60093	-1.80348	0.679832	-2.20327
30	Q-Q-P-R-K-I-R-P-E-G-R-E-G-E-Q	-1.49654	-1.85575	-0.43065	-1.56617	-1.59888	1.073113	-2.19143
31	K-I-R-P-E-G-R-E-G-E-Q-E-W-G-T	-1.78499	-2.21226	-0.92722	-2.06017	-2.19764	-0.78581	-2.77595
32	E-G-R-E-G-E-Q-E-W-G-T-P-G-S-H	-0.80859	-0.92786	0.083089	-1.02481	-0.89604	-0.74084	-1.51788
33	G-E-Q-E-W-G-T-P-G-S-H-V-R-E-E	-1.12135	-1.32287	-0.59095	-1.59858	-1.42527	-0.84743	-2.07836
34	W-G-T-P-G-S-H-V-R-E-E-T-S-R-N	-0.53332	-1.14455	-0.05914	-1.20476	-1.06486	0.978097	-1.55899
35	G-S-H-V-R-E-E-T-S-R-N-N-P-F-Y	-0.01043	-0.09614	0.440311	-0.50006	-0.32543	1.134864	-1.18208
36	R-E-E-T-S-R-N-N-P-F-Y-F-P-S-R	-0.82036	-1.40932	-0.1763	-1.5243	-1.43969	2.120055	-1.95952
37	S-R-N-N-P-F-Y-F-P-S-R-R-F-S-T	-3.44752	-3.95816	-3.20639	-4.74483	-4.20258	6.11492	-5.01601
38	P-F-Y-F-P-S-R-R-F-S-T-R-Y-G-N	-5.06892	-5.32476	-3.69021	-5.54657	-5.54197	1.857467	-5.87802
39	P-S-R-R-F-S-T-R-Y-G-N-Q-N-G-R	-1.46638	-1.85275	-0.93971	-2.6365	-2.08728	5.462969	-2.87961
40	F-S-T-R-Y-G-N-Q-N-G-R-I-R-V-L	-5.51754	-4.9093	-3.59513	-6.07071	-5.54662	1.951398	-6.1746

41	Y-G-N-Q-N-G-R-I-R-V-L-Q-R-F-D	-3.80173	-4.28143	-3.51195	-4.77467	-4.53855	1.637606	-4.99667
42	N-G-R-I-R-V-L-Q-R-F-D-Q-R-S-R	-1.05402	-1.4463	-0.37499	-2.09876	-1.85343	8.269298	-2.47961
43	R-V-L-Q-R-F-D-Q-R-S-R-Q-F-Q-N	-3.77368	-4.26005	-2.81649	-3.46217	-3.76985	-3.08607	-3.81267
44	R-F-D-Q-R-S-R-Q-F-Q-N-L-Q-N-H	-3.51472	-3.76369	-2.65536	-3.32275	-3.37184	-1.93313	-3.75799
45	R-S-R-Q-F-Q-N-L-Q-N-H-R-I-V-Q	-4.65688	-5.20262	-3.67376	-4.37583	-4.3852	-2.9254	-4.83482
46	F-Q-N-L-Q-N-H-R-I-V-Q-I-E-A-K	-4.96953	-5.19897	-4.1249	-4.81221	-5.01963	-3.5504	-5.15906
47	Q-N-H-R-I-V-Q-I-E-A-K-P-N-T-L	-1.84435	-2.61608	-1.24135	-1.99534	-1.85301	-0.89474	-2.3293
48	I-V-Q-I-E-A-K-P-N-T-L-V-L-P-K	-2.79282	-3.19444	-2.12935	-2.79919	-2.76277	-1.42306	-3.56109
49	E-A-K-P-N-T-L-V-L-P-K-H-A-D-A	-2.58609	-2.53274	-1.5097	-2.47911	-2.36149	-0.87698	-2.99702
50	N-T-L-V-L-P-K-H-A-D-A-D-N-I-L	-2.03782	-2.43916	-1.10017	-1.73145	-1.60425	0.241955	-2.379
51	L-P-K-H-A-D-A-D-N-I-L-V-I-Q-Q	-2.81641	-3.7833	-2.41846	-3.1225	-3.30017	1.703604	-3.77154
52	A-D-A-D-N-I-L-V-I-Q-Q-G-Q-A-T	-4.19235	-4.3313	-3.26534	-4.09327	-4.33864	-1.00651	-4.652
53	N-I-L-V-I-Q-Q-G-Q-A-T-V-T-V-A	-2.16774	-2.13731	-1.27606	-2.39048	-2.42897	0.209174	-2.64583
54	I-Q-Q-G-Q-A-T-V-T-V-A-N-G-N-N	-2.68989	-2.60579	-1.90558	-2.87914	-2.90811	0.692469	-3.47579
55	Q-A-T-V-T-V-A-N-G-N-N-R-K-S-F	-4.75025	-4.47719	-3.99292	-4.88241	-4.93557	-0.46567	-5.21174
56	T-V-A-N-G-N-N-R-K-S-F-N-L-D-E	-1.86184	-2.27533	-1.75553	-2.35259	-2.23456	-0.76586	-2.87459
57	G-N-N-R-K-S-F-N-L-D-E-G-H-A-L	-4.56008	-4.34359	-3.16136	-4.98688	-4.90878	-1.51123	-5.22503
58	K-S-F-N-L-D-E-G-H-A-L-R-I-P-S	-0.91191	-1.43548	-0.23171	-1.45091	-1.47296	1.616476	-1.88548
59	L-D-E-G-H-A-L-R-I-P-S-G-F-I-S	-0.43649	-0.7397	0.05526	-0.8411	-0.93607	4.109905	-1.36711
60	H-A-L-R-I-P-S-G-F-I-S-Y-I-L-N	-0.78396	-1.15791	-0.0508	-1.23558	-1.25632	2.256282	-1.51057
61	I-P-S-G-F-I-S-Y-I-L-N-R-H-D-N	-1.84747	-2.13996	-1.55845	-2.59927	-2.38757	0.955149	-2.88912
62	F-I-S-Y-I-L-N-R-H-D-N-Q-N-L-R	-3.59099	-3.73401	-3.22023	-4.57768	-4.23679	1.134951	-4.7398
63	I-L-N-R-H-D-N-Q-N-L-R-V-A-K-I	-2.88255	-3.44661	-2.73926	-3.77429	-3.67302	0.770051	-4.28396
64	H-D-N-Q-N-L-R-V-A-K-I-S-M-P-V	-1.97813	-2.0283	-0.98189	-2.95385	-2.59865	0.826378	-2.94594
65	N-L-R-V-A-K-I-S-M-P-V-N-T-P-G	-0.06683	-0.34011	0.182529	-0.76292	-0.63256	1.861246	-1.14492
66	A-K-I-S-M-P-V-N-T-P-G-Q-F-E-D	-1.38379	-1.52475	-1.38817	-2.40464	-2.37183	0.172457	-2.86822
67	M-P-V-N-T-P-G-Q-F-E-D-F-F-P-A	-5.85822	-5.20927	-4.92427	-5.43	-5.6348	-3.79248	-6.03225
68	T-P-G-Q-F-E-D-F-F-P-A-S-S-R-D	-5.12523	-4.7924	-3.86626	-4.76564	-5.0775	-2.74772	-5.17944
69	F-E-D-F-F-P-A-S-S-R-D-Q-S-S-Y	-8.47565	-8.33525	-7.09172	-8.10856	-8.22756	-5.29794	-8.77113
70	F-P-A-S-S-R-D-Q-S-S-Y-L-Q-G-F	-4.33994	-4.31495	-3.36856	-4.21691	-4.08812	-2.66787	-4.6224
71	S-R-D-Q-S-S-Y-L-Q-G-F-S-R-N-T	-2.16972	-2.79448	-1.40468	-2.20131	-2.2241	0.762336	-2.67811
72	S-S-Y-L-Q-G-F-S-R-N-T-L-E-A-A	-3.93724	-4.48038	-2.8885	-3.82911	-4.04678	-1.6196	-4.547
73	Q-G-F-S-R-N-T-L-E-A-A-F-N-A-E	-2.27504	-2.51554	-1.50664	-2.20462	-2.19394	-1.11055	-2.65728
74	R-N-T-L-E-A-A-F-N-A-E-F-N-E-I	-1.71356	-2.41752	-1.04764	-1.94978	-1.85634	0.944732	-2.3838
75	E-A-A-F-N-A-E-F-N-E-I-R-R-V-L	-5.03462	-5.35444	-4.39359	-5.18735	-5.4194	-0.20395	-5.56858
76	N-A-E-F-N-E-I-R-R-V-L-L-E-E-N	-4.20345	-4.0091	-3.27078	-4.39007	-4.33249	-1.61252	-4.49492
77	N-E-I-R-R-V-L-L-E-E-N-A-G-G-E	-1.80583	-1.47294	-0.82549	-1.65952	-1.92247	2.170454	-2.27343
78	R-V-L-L-E-E-N-A-G-G-E-Q-E-E-R	-2.48007	-2.28278	-1.70954	-2.53663	-2.44476	-0.22067	-3.09094
79	E-E-N-A-G-G-E-Q-E-E-R-G-Q-R-R	-3.02944	-3.03866	-2.4653	-3.39789	-3.43389	0.593273	-3.55882
80	G-G-E-Q-E-E-R-G-Q-R-R-W-S-T-R	-1.35112	-1.57238	-1.23478	-1.84748	-1.83559	-1.55267	-2.0053
81	E-E-R-G-Q-R-R-W-S-T-R-S-S-E-N	-1.49783	-1.70226	-0.9506	-2.01922	-2.27405	0.053035	-2.4349
82	Q-R-R-W-S-T-R-S-S-E-N-N-E-G-V	-0.57615	-1.05202	-0.32236	-1.23418	-1.45929	-0.29071	-1.84418
83	S-T-R-S-S-E-N-N-E-G-V-I-V-K-V	-0.05901	-0.11086	0.316488	-0.66801	-0.63321	1.758435	-1.00417
84	S-E-N-N-E-G-V-I-V-K-V-S-K-E-H	0.28641	-0.1019	0.471391	-0.3614	-0.40723	1.853793	-0.85168
85	E-G-V-I-V-K-V-S-K-E-H-V-E-E-L	-0.16239	-0.48878	0.110208	-1.08097	-0.87345	2.711468	-1.40266

86	V-K-V-S-K-E-H-V-E-E-L-T-K-H-A	-0.1189	-0.44542	0.040216	-0.89944	-0.81816	1.27644	-1.14074
87	K-E-H-V-E-E-L-T-K-H-A-K-S-V-S	0.543002	0.035546	0.846052	-0.09278	-0.12561	2.9907	-0.68633
88	E-E-L-T-K-H-A-K-S-V-S-K-K-G-S	-2.39508	-1.71374	-1.48004	-2.88311	-2.68003	1.902759	-3.7943
89	K-H-A-K-S-V-S-K-K-G-S-E-E-E-G	-0.23525	-0.47456	0.065399	-1.13809	-0.94217	0.072079	-1.13614
90	S-V-S-K-K-G-S-E-E-E-G-D-I-T-N	0.087879	-0.37212	-0.06961	-1.00511	-0.35301	1.492912	-1.13601
91	K-G-S-E-E-E-G-D-I-T-N-P-I-N-L	-6.08251	-5.91936	-5.24423	-5.58883	-5.75125	-3.95706	-6.28482
92	E-E-G-D-I-T-N-P-I-N-L-R-E-G-E	-3.58016	-3.68533	-3.07699	-3.39914	-3.65236	-1.84062	-3.90622
93	I-T-N-P-I-N-L-R-E-G-E-P-D-L-S	-2.79064	-3.34776	-2.19172	-2.78314	-2.87749	-0.89131	-3.29161
94	I-N-L-R-E-G-E-P-D-L-S-N-N-F-G	-6.46538	-6.59322	-5.76331	-6.44506	-6.59334	-5.55489	-6.89539
95	E-G-E-P-D-L-S-N-N-F-G-K-L-F-E	-1.73921	-2.02205	-0.84639	-1.65204	-1.75084	0.267964	-1.89913
96	D-L-S-N-N-F-G-K-L-F-E-V-K-P-D	-3.22114	-3.63672	-2.60546	-3.10983	-3.162	-0.06525	-3.5238
97	N-F-G-K-L-F-E-V-K-P-D-K-K-N-P	-1.31596	-1.62981	-0.25386	-1.27443	-1.1992	1.523191	-1.63908
98	L-F-E-V-K-P-D-K-K-N-P-Q-L-Q-D	-1.17964	-1.62633	-0.52397	-1.20924	-1.17964	1.809375	-1.65344
99	K-P-D-K-K-N-P-Q-L-Q-D-L-D-M-M	-1.48463	-1.8325	-0.78873	-1.67927	-1.74847	0.81194	-1.93796
100	K-N-P-Q-L-Q-D-L-D-M-M-L-T-C-V	-1.1888	-1.33911	-0.83262	-1.49981	-1.53864	0.551244	-1.77933
101	L-Q-D-L-D-M-M-L-T-C-V-E-I-K-E	-1.07302	-1.24302	-0.4837	-1.36357	-1.35759	0.565644	-1.5592
102	D-M-M-L-T-C-V-E-I-K-E-G-A-L-M	-0.75028	-0.82139	-0.13536	-1.16192	-0.99911	-0.0593	-1.44571
103	T-C-V-E-I-K-E-G-A-L-M-L-P-H-F	-0.36114	-0.85343	0.038987	-0.9403	-0.89087	0.028327	-1.12037
104	I-K-E-G-A-L-M-L-P-H-F-N-S-K-A	-0.55012	-0.78821	-0.50482	-0.8001	-1.06928	0.873289	-1.20742
105	A-L-M-L-P-H-F-N-S-K-A-M-V-I-V	-0.42655	-0.58278	-0.23174	-1.01486	-0.9662	0.99738	-1.06592
106	P-H-F-N-S-K-A-M-V-I-V-V-V-N-K	-0.0925	-0.36752	0.200043	-0.77501	-0.59099	2.871109	-0.95162
107	S-K-A-M-V-I-V-V-V-N-K-G-T-G-N	0.173235	-0.35064	0.432994	-0.73092	-0.69294	1.195947	-1.14421
108	V-I-V-V-V-N-K-G-T-G-N-L-E-L-V	0.537866	0.248393	0.824026	-0.26237	-0.21496	4.333803	-0.61314
109	V-N-K-G-T-G-N-L-E-L-V-A-V-R-K	0.413391	-0.1447	0.535107	-0.62545	-0.40154	2.190412	-0.8985
110	T-G-N-L-E-L-V-A-V-R-K-E-Q-Q-Q	0.18656	-0.31804	0.49589	-0.76449	-0.32634	1.811087	-0.96414
111	E-L-V-A-V-R-K-E-Q-Q-Q-R-G-R-R	-0.68506	-0.6671	-0.12759	-1.34106	-1.52936	1.899978	-1.8767
112	V-R-K-E-Q-Q-Q-R-G-R-R-E-E-E-E	-1.68546	-1.39896	-1.15009	-2.14635	-2.04733	-0.85528	-2.21273
113	Q-Q-Q-R-G-R-R-E-E-E-E-D-E-D-E	0.148499	-0.35106	0.042775	-0.94841	-0.77974	0.284108	-1.25717
114	G-R-R-E-E-E-E-D-E-D-E-E-E-E-G	-0.12566	-0.38224	-0.02598	-1.18015	-0.95077	0.555771	-1.46751
115	E-E-E-D-E-D-E-E-E-E-G-S-N-R-E	-4.43209	-4.32553	-3.83032	-4.17066	-4.47604	-2.46544	-4.92847
116	E-D-E-E-E-E-G-S-N-R-E-V-R-R-Y	-5.17861	-4.79219	-4.63093	-4.8571	-5.13206	-3.78025	-5.18906
117	E-E-G-S-N-R-E-V-R-R-Y-T-A-R-L	-4.67646	-4.65452	-3.95036	-4.76744	-4.70542	-1.22555	-4.86127
118	N-R-E-V-R-R-Y-T-A-R-L-K-E-G-D	-6.32906	-6.18001	-5.61922	-6.39417	-6.36808	-3.57857	-6.41153
119	R-R-Y-T-A-R-L-K-E-G-D-V-F-I-M	-4.64388	-4.75712	-3.79146	-4.41361	-4.24739	-1.12305	-4.74242
120	A-R-L-K-E-G-D-V-F-I-M-P-A-A-H	-1.77888	-2.24825	-1.32342	-1.89917	-2.05783	-0.4094	-2.10347
121	E-G-D-V-F-I-M-P-A-A-H-P-V-A-I	-1.63326	-1.51474	-0.89538	-1.24835	-1.39583	1.632756	-1.66071
122	F-I-M-P-A-A-H-P-V-A-I-N-A-S-S	-0.24594	-0.73	-0.51764	-0.94672	-0.92693	-0.6024	-1.11852
123	A-A-H-P-V-A-I-N-A-S-S-E-L-H-L	-0.0888	-0.42306	0.010493	-0.77237	-0.70429	0.041462	-0.63688
124	V-A-I-N-A-S-S-E-L-H-L-L-G-F-G	-0.69734	-0.543	-0.31077	-1.19915	-1.37762	-0.18655	-1.59389
125	A-S-S-E-L-H-L-L-G-F-G-I-N-A-E	-1.77673	-1.84522	-1.55784	-2.20931	-2.2245	-0.80088	-2.3461
126	L-H-L-L-G-F-G-I-N-A-E-N-N-H-R	-1.75273	-1.57223	-0.84303	-1.66117	-1.55857	2.041928	-2.294
127	G-F-G-I-N-A-E-N-N-H-R-I-F-L-A	-1.26419	-1.58705	-1.01378	-1.83206	-1.6283	1.645773	-2.05492
128	N-A-E-N-N-H-R-I-F-L-A-G-D-K-D	-0.79452	-0.83485	-0.7815	-1.2169	-1.10286	1.319537	-1.42178
129	N-H-R-I-F-L-A-G-D-K-D-N-V-I-D	-1.44956	-1.44486	-1.18522	-1.84764	-2.06453	0.939484	-2.30841
130	F-L-A-G-D-K-D-N-V-I-D-Q-I-E-K	-1.969	-1.85691	-1.66995	-2.41359	-2.23676	1.542752	-2.67637

131	D-K-D-N-V-I-D-Q-I-E-K-Q-A-K-D	-0.38422	-0.96466	-0.75624	-1.27404	-1.36663	1.253026	-1.39168
132	V-I-D-Q-I-E-K-Q-A-K-D-L-A-F-P	0.700561	0.343285	0.708647	-0.20513	-0.14982	1.513708	-0.46916
133	I-E-K-Q-A-K-D-L-A-F-P-G-S-G-E	0.745155	0.610842	1.853715	-0.15906	-0.04367	0.885985	-0.4803
134	A-K-D-L-A-F-P-G-S-G-E-Q-V-E-K	0.689793	0.549704	4.461783	-0.02356	-0.01489	3.67867	-0.17478
135	A-F-P-G-S-G-E-Q-V-E-K-L-I-K-N	-0.53511	-0.76289	-0.17308	-1.17784	-1.33922	1.009453	-1.58414
136	S-G-E-Q-V-E-K-L-I-K-N-Q-K-E-S	-0.21969	-0.51256	0.012519	-1.04923	-0.62634	-0.57752	-1.02176
137	V-E-K-L-I-K-N-Q-K-E-S-H-F-V-S	-0.35575	-0.21945	-0.27371	-1.24861	-0.956	-1.15982	-1.51356
138	I-K-N-Q-K-E-S-H-F-V-S-A-R-P-Q	0.072311	-0.1279	0.000766	-1.05071	-0.89162	-0.30476	-1.09003
139	K-E-S-H-F-V-S-A-R-P-Q-S-Q-S-Q	-2.97073	-2.73124	-2.51785	-2.53422	-3.16457	-1.61284	-2.98804
140	F-V-S-A-R-P-Q-S-Q-S-Q-S-P-S-S	-3.31237	-3.36941	-2.91323	-2.95238	-3.36365	-2.42809	-3.44926
141	R-P-Q-S-Q-S-Q-S-P-S-S-P-E-K-E	-8.82035	-8.58667	-7.81938	-8.4913	-8.71475	-6.16303	-8.08372
142	Q-S-Q-S-P-S-S-P-E-K-E-S-P-E-K	-3.73943	-3.92663	-3.46774	-3.93155	-3.96514	-1.72332	-4.17279
143	P-S-S-P-E-K-E-S-P-E-K-E-D-Q-E	-2.68931	-2.76318	-2.45208	-2.71261	-2.65349	-0.36627	-2.90363
144	E-K-E-S-P-E-K-E-D-Q-E-E-E-N-Q	-2.02813	-2.48894	-1.48227	-2.12817	-2.16613	-0.01414	-2.26965
145	P-E-K-E-D-Q-E-E-E-N-Q-G-G-K-G	-1.37895	-1.29748	-1.15837	-1.43399	-1.57587	-0.34633	-1.7935
146	D-Q-E-E-E-N-Q-G-G-K-G-P-L-L-S	-1.94893	-1.87866	-1.67339	-1.94198	-1.84066	-0.21488	-2.1844
147	E-N-Q-G-G-K-G-P-L-L-S-I-L-K-A	-2.61285	-2.91023	-2.471	-3.03415	-3.09521	-0.77711	-3.30461
148	Q-G-G-K-G-P-L-L-S-I-L-K-A-F-N	-1.60418	-1.7631	-1.47978	-2.18875	-1.87553	-0.31158	-2.13019
	m (blanks)	12537.95	10771.03	12268.53	14071.12	13118.96	11883.05	11332.93
	s (blanks)	353.6775	354.0388	363.7228	459.9209	431.5639	526.1645	436.8518

Table A6: Calculated Z-scores of Ara h 1 peptides of controls and derived maximum Z-score.

The calculated Z-scores of every peptide of Ara h 1 for each control and the maximum Z-score (Max.) of the controls are listed. Numbers written behind control sera represent the respective developed X-ray film.

	Control No. →	N_1	N_2	N_3	DLab71S1_1	DLab71S1_2	DLab71S1_3	Max.
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	K-S-S-P-Y-Q-K-K-T-E-N-P-C-A-Q	1.070546	0.580083	2.082704	0.059552	-0.05839	1.68182	2.082704
2	Y-Q-K-K-T-E-N-P-C-A-Q-R-C-L-Q	1.334444	0.563629	1.858645	0.212815	0.017896	1.723178	1.858645
3	T-E-N-P-C-A-Q-R-C-L-Q-S-C-Q-Q	1.981947	0.584197	1.996702	0.901739	-0.01071	2.168927	2.168927
4	C-A-Q-R-C-L-Q-S-C-Q-Q-E-P-D-D	1.190252	0.376458	1.654955	-0.26518	-0.15185	1.461243	1.654955
5	C-L-Q-S-C-Q-Q-E-P-D-D-L-K-Q-K	1.462312	0.701436	2.195865	0.516306	-0.01834	1.913885	2.195865
6	C-Q-Q-E-P-D-D-L-K-Q-K-A-C-E-S	0.287013	0.503981	2.200392	-0.27732	-0.06793	0.838574	2.200392
7	P-D-D-L-K-Q-K-A-C-E-S-R-C-T-K	0.586279	0.765197	1.770379	-0.13316	-0.17473	1.116592	1.770379
8	K-Q-K-A-C-E-S-R-C-T-K-L-E-Y-D	1.350768	0.650015	1.937858	0.540585	0.206712	1.801299	1.937858
9	C-E-S-R-C-T-K-L-E-Y-D-P-R-C-V	1.290914	0.222197	1.428632	0.481404	-0.1137	1.309597	1.428632
10	C-T-K-L-E-Y-D-P-R-C-V-Y-D-P-R	1.323562	0.273617	1.924278	0.508718	-0.04886	1.54396	1.924278
11	E-Y-D-P-R-C-V-Y-D-P-R-G-H-T-G	0.371351	0.263333	1.233995	0.422224	-0.20716	1.072936	1.233995
12	R-C-V-Y-D-P-R-G-H-T-G-T-T-N-Q	2.349228	0.275674	1.75001	0.38884	-0.22051	0.742071	2.349228
13	D-P-R-G-H-T-G-T-T-N-Q-R-S-P-P	1.535768	0.724061	1.512371	1.102043	-0.09082	1.465839	1.535768
14	H-T-G-T-T-N-Q-R-S-P-P-G-E-R-T	1.122237	0.563629	1.591584	0.683226	0.021711	0.944267	1.591584
15	T-N-Q-R-S-P-P-G-E-R-T-R-G-R-Q	2.648494	0.861868	2.075914	1.457128	0.086556	1.872527	2.648494
16	S-P-P-G-E-R-T-R-G-R-Q-P-G-D-Y	2.259448	0.724061	1.295102	0.672604	0.212433	0.900611	2.259448
17	E-R-T-R-G-R-Q-P-G-D-Y-D-D-D-R	1.628269	0.818674	1.000883	0.985199	0.485167	1.155653	1.628269
18	G-R-Q-P-G-D-Y-D-D-D-R-R-Q-P-R	2.667538	1.244436	2.827305	2.099011	0.925735	2.649141	2.827305

19	G-D-Y-D-D-D-R-R-Q-P-R-R-E-E-G	2.683862	1.546789	4.73973	1.939678	1.043983	4.069105	4.73973
20	D-D-R-R-Q-P-R-R-E-E-G-G-R-W-G	0.986207	1.371959	2.852201	-0.13772	0.797951	3.104081	3.104081
21	Q-P-R-R-E-E-G-G-R-W-G-P-A-G-P	0.869221	1.2033	3.189421	0.38277	0.456558	3.395886	3.395886
22	E-E-G-G-R-W-G-P-A-G-P-R-E-R-E	1.990109	0.728174	2.734513	0.951815	0.410785	2.761728	2.761728
23	R-W-G-P-A-G-P-R-E-R-E-R-E-E-D	1.195693	0.62122	2.112126	0.469265	0.391712	2.132165	2.132165
24	A-G-P-R-E-R-E-R-E-E-D-W-R-Q-P	1.709887	0.528663	3.522115	1.275033	0.136144	2.922565	3.522115
25	E-R-E-R-E-E-D-W-R-Q-P-R-E-D-W	0.825692	0.431992	1.901646	0.012511	-0.02406	1.603699	1.901646
26	E-E-D-W-R-Q-P-R-E-D-W-R-R-P-S	0.967163	0.345606	1.575742	-0.23332	0.006453	2.2953	2.2953
27	R-Q-P-R-E-D-W-R-R-P-S-H-Q-Q-P	0.569955	0.512208	1.969543	0.276548	-0.0069	1.904695	1.969543
28	E-D-W-R-R-P-S-H-Q-Q-P-R-K-I-R	0.915472	0.497811	1.611953	0.064104	-0.12705	1.580723	1.611953
29	R-P-S-H-Q-Q-P-R-K-I-R-P-E-G-R	1.538489	0.345606	1.933331	0.190053	-0.05076	1.25675	1.933331
30	Q-Q-P-R-K-I-R-P-E-G-R-E-G-E-Q	0.959001	0.549231	1.768116	1.007961	0.046505	1.504899	1.768116
31	K-I-R-P-E-G-R-E-G-E-Q-E-W-G-T	1.775181	0.85364	2.213971	1.337249	-0.12133	2.325169	2.325169
32	E-G-R-E-G-E-Q-E-W-G-T-P-G-S-H	0.414881	0.549231	1.215889	-0.04667	-0.1404	1.08902	1.215889
33	G-E-Q-E-W-G-T-P-G-S-H-V-R-E-E	1.323562	0.736402	1.747747	-0.02846	-0.05839	1.867932	1.867932
34	W-G-T-P-G-S-H-V-R-E-E-T-S-R-N	0.303336	0.092617	1.424106	0.736337	-0.43602	1.254453	1.424106
35	G-S-H-V-R-E-E-T-S-R-N-N-P-F-Y	0.689662	0.201628	0.754191	0.498096	-0.22814	0.691522	0.754191
36	R-E-E-T-S-R-N-N-P-F-Y-F-P-S-R	1.992829	0.450504	2.03065	1.478372	0.174289	1.236071	2.03065
37	S-R-N-N-P-F-Y-F-P-S-R-R-F-S-T	5.409904	1.773039	4.502092	3.373673	0.710219	2.196499	5.409904
38	P-F-Y-F-P-S-R-R-F-S-T-R-Y-G-N	6.457335	1.038754	3.092103	1.894154	0.344032	1.71169	6.457335
39	P-S-R-R-F-S-T-R-Y-G-N-Q-N-G-R	2.784524	0.839243	3.442902	1.542105	0.54429	2.134462	3.442902
40	F-S-T-R-Y-G-N-Q-N-G-R-I-R-V-L	7.129323	1.51388	4.196556	3.109635	0.830374	3.25343	7.129323
41	Y-G-N-Q-N-G-R-I-R-V-L-Q-R-F-D	5.894171	1.275289	2.78883	2.126325	0.710219	2.607783	5.894171
42	N-G-R-I-R-V-L-Q-R-F-D-Q-R-S-R	3.287835	1.828573	2.739039	1.883532	0.464187	1.849551	3.287835
43	R-V-L-Q-R-F-D-Q-R-S-R-Q-F-Q-N	0.450249	1.287629	3.182632	0.381252	1.22517	4.098974	4.098974
44	R-F-D-Q-R-S-R-Q-F-Q-N-L-Q-N-H	1.818711	1.102516	3.698647	0.778825	0.813209	3.326956	3.698647
45	R-S-R-Q-F-Q-N-L-Q-N-H-R-I-V-Q	2.542391	1.232095	3.612644	2.479892	0.981045	5.038724	5.038724
46	F-Q-N-L-Q-N-H-R-I-V-Q-I-E-A-K	5.388139	0.617106	3.157736	2.79097	0.490888	3.726751	5.388139
47	Q-N-H-R-I-V-Q-I-E-A-K-P-N-T-L	0.929075	0.39497	1.96728	0.135425	0.227691	2.251644	2.251644
48	I-V-Q-I-E-A-K-P-N-T-L-V-L-P-K	3.089231	0.425822	3.101155	1.794002	0.326867	2.823765	3.101155
49	E-A-K-P-N-T-L-V-L-P-K-H-A-D-A	1.36165	0.538947	2.562508	0.757581	0.191454	2.676714	2.676714
50	N-T-L-V-L-P-K-H-A-D-A-D-N-I-L	1.144002	0.586254	1.697956	0.300827	0.126608	2.074723	2.074723
51	L-P-K-H-A-D-A-D-N-I-L-V-I-Q-Q	3.480998	0.62122	2.809199	2.795522	0.347846	3.232751	3.480998
52	A-D-A-D-N-I-L-V-I-Q-Q-G-Q-A-T	4.514826	0.499867	2.404082	2.109633	0.349754	2.633058	4.514826
53	N-I-L-V-I-Q-Q-G-Q-A-T-V-T-V-A	2.575038	0.518379	2.118916	0.678673	0.557641	2.336658	2.575038
54	I-Q-Q-G-Q-A-T-V-T-V-A-N-G-N-N	3.102834	0.62739	2.485558	1.435883	0.094185	2.139058	3.102834
55	Q-A-T-V-T-V-A-N-G-N-N-R-K-S-F	5.170491	0.806334	4.404773	2.71358	0.361197	5.443114	5.443114
56	T-V-A-N-G-N-N-R-K-S-F-N-L-D-E	2.732833	0.508095	2.218498	2.162744	0.263928	2.099997	2.732833
57	G-N-N-R-K-S-F-N-L-D-E-G-H-A-L	3.908132	0.573913	2.17097	1.862288	0.471816	5.323635	5.323635
58	K-S-F-N-L-D-E-G-H-A-L-R-I-P-S	0.572676	0.119356	1.297365	0.520858	-0.09654	1.709392	1.709392
59	L-D-E-G-H-A-L-R-I-P-S-G-F-I-S	0.746794	0.347663	1.18873	0.02465	-0.20716	0.542173	1.18873
60	H-A-L-R-I-P-S-G-F-I-S-Y-I-L-N	0.746794	0.619163	1.654955	1.149084	0.014082	1.491113	1.654955
61	I-P-S-G-F-I-S-Y-I-L-N-R-H-D-N	3.170849	0.512208	1.89712	1.058037	0.187639	0.969541	3.170849
62	F-I-S-Y-I-L-N-R-H-D-N-Q-N-L-R	5.140564	0.880379	2.684722	2.501136	0.20099	1.647355	5.140564
63	I-L-N-R-H-D-N-Q-N-L-R-V-A-K-I	4.604606	0.652072	2.152864	1.936643	0.246763	3.478602	4.604606

64	H-D-N-Q-N-L-R-V-A-K-I-S-M-P-V	3.875485	0.979106	2.487821	2.668056	0.540476	3.005281	3.875485
65	N-L-R-V-A-K-I-S-M-P-V-N-T-P-G	1.930256	0.645902	1.89033	1.305382	0.481352	1.693309	1.930256
66	A-K-I-S-M-P-V-N-T-P-G-Q-F-E-D	3.674161	1.388414	3.144157	2.006446	0.832281	2.764025	3.674161
67	M-P-V-N-T-P-G-Q-F-E-D-F-F-P-A	5.311962	1.489198	5.405119	3.699925	1.593264	6.327719	6.327719
68	T-P-G-Q-F-E-D-F-F-P-A-S-S-R-D	5.023578	1.256777	5.178796	2.786418	1.175582	5.374184	5.374184
69	F-E-D-F-F-P-A-S-S-R-D-Q-S-S-Y	7.006896	2.019857	5.280641	2.828906	2.594557	9.078036	9.078036
70	F-P-A-S-S-R-D-Q-S-S-Y-L-Q-G-F	4.427767	0.89889	3.368216	3.871398	0.50996	4.549319	4.549319
71	S-R-D-Q-S-S-Y-L-Q-G-F-S-R-N-T	2.322022	0.752856	2.24113	0.754546	0.18001	2.483709	2.483709
72	S-S-Y-L-Q-G-F-S-R-N-T-L-E-A-A	4.53115	0.884493	3.311635	2.54059	0.714034	4.234537	4.53115
73	Q-G-F-S-R-N-T-L-E-A-A-F-N-A-E	2.22136	0.378515	2.467452	0.78186	0.159031	2.143653	2.467452
74	R-N-T-L-E-A-A-F-N-A-E-F-N-E-I	1.884005	0.489583	1.89033	0.44195	0.138052	2.132165	2.132165
75	E-A-A-F-N-A-E-F-N-E-I-R-R-V-L	5.458875	1.049038	3.064944	2.097493	0.50996	2.20569	5.458875
76	N-A-E-F-N-E-I-R-R-V-L-L-E-E-N	4.520267	0.545117	2.759408	0.957885	0.216248	2.950137	4.520267
77	N-E-I-R-R-V-L-L-E-E-N-A-G-G-E	1.737093	0.707606	1.747747	1.989754	0.141866	1.863337	1.989754
78	R-V-L-L-E-E-N-A-G-G-E-Q-E-E-R	1.514004	0.631504	2.31129	1.561832	0.33831	2.695095	2.695095
79	E-E-N-A-G-G-E-Q-E-E-R-G-Q-R-R	2.433567	0.489583	2.118916	1.479889	0.267743	3.54983	3.54983
80	G-G-E-Q-E-E-R-G-Q-R-R-W-S-T-R	2.058124	0.384685	1.451264	0.156669	0.035061	2.019579	2.058124
81	E-E-R-G-Q-R-R-W-S-T-R-S-S-E-N	1.843196	0.364117	1.360735	0.534515	-0.10989	2.332062	2.332062
82	Q-R-R-W-S-T-R-S-S-E-N-N-E-G-V	0.221718	0.154322	1.623269	0.375182	-0.1404	1.68182	1.68182
83	S-T-R-S-S-E-N-N-E-G-V-I-V-K-V	-0.5727	0.345606	0.56408	0.839524	-0.28345	0.356062	0.839524
84	S-E-N-N-E-G-V-I-V-K-V-S-K-E-H	0.197233	0.043253	0.822088	0.193088	-0.22432	0.333085	0.822088
85	E-G-V-I-V-K-V-S-K-E-H-V-E-E-L	0.175468	0.285958	1.482949	0.127837	0.094185	0.581234	1.482949
86	V-K-V-S-K-E-H-V-E-E-L-T-K-H-A	0.763118	0.590367	1.566689	0.575486	0.046505	1.236071	1.566689
87	K-E-H-V-E-E-L-T-K-H-A-K-S-V-S	-0.06939	0.545117	1.068779	0.715092	-0.01643	0.788025	1.068779
88	E-E-L-T-K-H-A-K-S-V-S-K-K-G-S	4.346149	0.85364	2.772988	2.913884	0.864704	3.513067	4.346149
89	K-H-A-K-S-V-S-K-K-G-S-E-E-E-G	2.033638	0.715833	1.702482	1.05045	0.454651	1.169439	2.033638
90	S-V-S-K-K-G-S-E-E-E-G-D-I-T-N	1.835035	1.026413	1.503319	1.64074	0.382176	2.072425	2.072425
91	K-G-S-E-E-E-G-D-I-T-N-P-I-N-L	2.964084	1.415152	4.698992	1.930573	1.351047	6.532212	6.532212
92	E-E-G-D-I-T-N-P-I-N-L-R-E-G-E	3.429307	1.184788	3.594539	1.187021	1.167953	4.018556	4.018556
93	I-T-N-P-I-N-L-R-E-G-E-P-D-L-S	0.303336	0.913288	3.246002	0.21585	0.828467	2.931756	3.246002
94	I-N-L-R-E-G-E-P-D-L-S-N-N-F-G	6.968808	1.084004	3.77786	3.032245	0.89522	4.85491	6.968808
95	E-G-E-P-D-L-S-N-N-F-G-K-L-F-E	1.337165	0.551288	1.906173	0.133907	0.260114	1.851848	1.906173
96	D-L-S-N-N-F-G-K-L-F-E-V-K-P-D	1.571136	0.48547	2.560245	0.794	0.502332	3.368314	3.368314
97	N-F-G-K-L-F-E-V-K-P-D-K-K-N-P	0.784883	0.224253	1.426369	0.373665	0.036969	1.270536	1.426369
98	L-F-E-V-K-P-D-K-K-N-P-Q-L-Q-D	0.265248	0.349719	1.372051	0.176396	0.103721	1.323383	1.372051
99	K-P-D-K-K-N-P-Q-L-Q-D-L-D-M-M	0.452969	0.318867	1.713798	0.083831	0.164753	1.44516	1.713798
100	K-N-P-Q-L-Q-D-L-D-M-M-L-T-C-V	0.216277	0.304469	1.573478	-0.15593	-0.01643	1.036174	1.573478
101	L-Q-D-L-D-M-M-L-T-C-V-E-I-K-E	0.205395	0.31681	1.317734	0.177913	-0.07937	1.378527	1.378527
102	D-M-M-L-T-C-V-E-I-K-E-G-A-L-M	-0.20814	0.658242	0.939775	-0.08764	0.092278	1.024685	1.024685
103	T-C-V-E-I-K-E-G-A-L-M-L-P-H-F	-0.30336	0.335322	0.946565	-0.12406	0.006453	0.719094	0.946565
104	I-K-E-G-A-L-M-L-P-H-F-N-S-K-A	1.051502	0.306526	1.141202	0.30538	0.069391	0.95116	1.141202
105	A-L-M-L-P-H-F-N-S-K-A-M-V-I-V	0.403999	0.279788	0.616134	0.103558	0.025525	1.079829	1.079829
106	P-H-F-N-S-K-A-M-V-I-V-V-V-N-K	-0.66248	0.133753	0.889985	0.003406	-0.15375	0.838574	0.889985
107	S-K-A-M-V-I-V-V-V-N-K-G-T-G-N	0.915472	0.121412	0.751928	0.674121	-0.26437	0.443373	0.915472
108	V-I-V-V-V-N-K-G-T-G-N-L-E-L-V	0.518264	0.047367	0.55729	0.297792	-0.05458	0.590425	0.590425

109	V-N-K-G-T-G-N-L-E-L-V-A-V-R-K	-0.05306	0.183117	0.55729	0.252269	-0.04123	0.994815	0.994815
110	T-G-N-L-E-L-V-A-V-R-K-E-Q-Q-Q	0.959001	0.370288	1.396947	0.577004	0.193361	0.689225	1.396947
111	E-L-V-A-V-R-K-E-Q-Q-Q-R-G-R-R	2.243125	0.594481	1.550846	2.130877	0.14568	1.417588	2.243125
112	V-R-K-E-Q-Q-Q-R-G-R-R-E-E-E-E	3.048422	0.736402	1.858645	1.982167	0.40697	2.612379	3.048422
113	Q-Q-Q-R-G-R-R-E-E-E-E-D-E-D-E	1.772461	0.637674	2.014807	1.71206	0.607229	2.028769	2.028769
114	G-R-R-E-E-E-E-D-E-D-E-E-E-E-G	2.232242	0.773424	1.247574	1.017066	0.7579	2.102295	2.232242
115	E-E-E-D-E-D-E-E-E-E-G-S-N-R-E	3.821073	1.334936	4.843839	2.780348	1.705791	4.983579	4.983579
116	E-D-E-E-E-E-G-S-N-R-E-V-R-R-Y	3.660558	1.262948	3.859336	1.448023	1.232799	5.498259	5.498259
117	E-E-G-S-N-R-E-V-R-R-Y-T-A-R-L	3.774823	0.80839	5.106373	2.432851	0.838003	3.924351	5.106373
118	N-R-E-V-R-R-Y-T-A-R-L-K-E-G-D	6.737557	0.826902	5.16069	3.516313	0.956251	6.750491	6.750491
119	R-R-Y-T-A-R-L-K-E-G-D-V-F-I-M	4.680783	0.524549	2.417661	1.780345	0.529033	2.566425	4.680783
120	A-R-L-K-E-G-D-V-F-I-M-P-A-A-H	0.374072	0.514265	2.053282	0.095971	0.273465	1.895504	2.053282
121	E-G-D-V-F-I-M-P-A-A-H-P-V-A-I	0.684221	0.127583	1.537267	0.338764	0.134237	1.491113	1.537267
122	F-I-M-P-A-A-H-P-V-A-I-N-A-S-S	-0.14012	0.300356	0.43055	0.161221	-0.17664	1.066043	1.066043
123	A-A-H-P-V-A-I-N-A-S-S-E-L-H-L	-0.33873	0.125526	0.659136	0.004924	-0.03932	0.815597	0.815597
124	V-A-I-N-A-S-S-E-L-H-L-L-G-F-G	0.202674	0.032969	0.91488	0.6984	-0.35211	1.47503	1.47503
125	A-S-S-E-L-H-L-L-G-F-G-I-N-A-E	1.260988	0.503981	2.254709	1.572454	0.170474	2.244751	2.254709
126	L-H-L-L-G-F-G-I-N-A-E-N-N-H-R	1.258267	0.510151	1.806591	1.28262	0.057948	1.511792	1.806591
127	G-F-G-I-N-A-E-N-N-H-R-I-F-L-A	1.494959	0.448447	1.894856	0.731784	0.128515	1.688713	1.894856
128	N-A-E-N-N-H-R-I-F-L-A-G-D-K-D	0.199954	0.102901	1.26568	0.696883	-0.00499	1.360146	1.360146
129	N-H-R-I-F-L-A-G-D-K-D-N-V-I-D	1.010693	0.179003	1.535004	0.854698	0.023618	2.173523	2.173523
130	F-L-A-G-D-K-D-N-V-I-D-Q-I-E-K	1.002531	0.037083	1.541793	0.957885	-0.15757	2.63076	2.63076
131	D-K-D-N-V-I-D-Q-I-E-K-Q-A-K-D	1.552092	-0.06987	0.980514	1.227992	-0.17283	1.213095	1.552092
132	V-I-D-Q-I-E-K-Q-A-K-D-L-A-F-P	0.31966	-0.03696	0.441866	0.13239	-0.3254	0.195224	0.441866
133	I-E-K-Q-A-K-D-L-A-F-P-G-S-G-E	0.360469	-0.09044	0.195174	0.139977	-0.07556	0.569746	0.569746
134	A-K-D-L-A-F-P-G-S-G-E-Q-V-E-K	0.749515	0.207799	0.525605	0.53148	0.027432	0.746667	0.749515
135	A-F-P-G-S-G-E-Q-V-E-K-L-I-K-N	2.101653	0.335322	0.923933	1.21737	0.206712	1.635867	2.101653
136	S-G-E-Q-V-E-K-L-I-K-N-Q-K-E-S	1.756137	0.477242	0.946565	1.907811	0.241042	1.082127	1.907811
137	V-E-K-L-I-K-N-Q-K-E-S-H-F-V-S	2.205036	0.769311	1.315471	2.03983	0.464187	1.605997	2.205036
138	I-K-N-Q-K-E-S-H-F-V-S-A-R-P-Q	2.161507	0.796049	1.152519	1.722682	0.889498	1.11889	2.161507
139	K-E-S-H-F-V-S-A-R-P-Q-S-Q-S-Q	1.862241	0.9318	2.582877	-0.04515	1.106922	3.338444	3.338444
140	F-V-S-A-R-P-Q-S-Q-S-Q-S-P-S-S	1.18209	0.855697	2.671143	0.270478	0.979138	3.634844	3.634844
141	R-P-Q-S-Q-S-Q-S-P-S-S-P-E-K-E	9.352054	2.063051	4.855155	3.540593	1.494089	9.156157	9.352054
142	Q-S-Q-S-P-S-S-P-E-K-E-S-P-E-K	2.379155	0.516322	2.415398	1.581559	0.54429	4.296575	4.296575
143	P-S-S-P-E-K-E-S-P-E-K-E-D-Q-E	2.90151	0.49164	2.322606	0.502649	0.361197	2.706583	2.90151
144	E-K-E-S-P-E-K-E-D-Q-E-E-E-N-Q	1.222899	0.36206	1.765853	0.71054	0.244856	2.214881	2.214881
145	P-E-K-E-D-Q-E-E-E-N-Q-G-G-K-G	0.472014	0.133753	1.541793	0.323589	0.014082	1.596806	1.596806
146	D-Q-E-E-E-N-Q-G-G-K-G-P-L-L-S	0.349587	0.382629	1.661744	0.393392	0.29063	2.06783	2.06783
147	E-N-Q-G-G-K-G-P-L-L-S-I-L-K-A	3.192614	0.493697	1.786222	1.80159	0.237227	2.66063	3.192614
148	Q-G-G-K-G-P-L-L-S-I-L-K-A-F-N	1.209296	0.343549	2.118916	0.828901	0.172382	1.932267	2.118916
	m (blanks)	13069.5	12623.97	12097.76	12973.76	11876.62	12228.03	
	s (blanks)	367.5659	486.1874	441.8473	658.9983	524.322	435.2225	

Table A7: Calculated Z-scores of Ara h 1 IgE inhibition experiments.

The calculated Z-scores of serum pool 1 (peanut patients 10, 12, 18 and 21) and serum pool 2 (peanut patients 6, 15, 17, 23) inhibited (+) and uninhibited (-) are listed. For inhibition 20.5 μg rAra h 1 was preincubated with both serum pools. As references, uninhibited serum pools preincubated with protein buffer without rAra h 1 were used. Identified candidate diagnostic peptides are highlighted in light blue.

	IgE inhibition experiment →	Pool 1 -	Pool 1 +	Pool 2-	Pool 2 +
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score
1	K-S-S-P-Y-Q-K-K-T-E-N-P-C-A-Q	3.201425	16.39898	1.118071	3.6101
2	Y-Q-K-K-T-E-N-P-C-A-Q-R-C-L-Q	3.004696	19.31349	0.345692	4.680069
3	T-E-N-P-C-A-Q-R-C-L-Q-S-C-Q-Q	12.18876	27.99487	0.491081	6.82209
4	C-A-Q-R-C-L-Q-S-C-Q-Q-E-P-D-D	3.406185	23.16972	1.530006	7.033583
5	C-L-Q-S-C-Q-Q-E-P-D-D-L-K-Q-K	2.775847	32.46468	1.414906	5.005123
6	C-Q-Q-E-P-D-D-L-K-Q-K-A-C-E-S	0.912937	9.100103	0.385068	1.082599
7	P-D-D-L-K-Q-K-A-C-E-S-R-C-T-K	2.950495	14.35437	0.860611	4.540462
8	K-Q-K-A-C-E-S-R-C-T-K-L-E-Y-D	2.029077	23.77553	1.193794	7.9952
9	C-E-S-R-C-T-K-L-E-Y-D-P-R-C-V	1.806251	22.37168	0.669788	4.580052
10	C-T-K-L-E-Y-D-P-R-C-V-Y-D-P-R	2.017032	23.0804	0.357808	1.781673
11	E-Y-D-P-R-C-V-Y-D-P-R-G-H-T-G	1.583424	13.22041	0.600122	1.968162
12	R-C-V-Y-D-P-R-G-H-T-G-T-T-N-Q	1.980898	15.19707	0.845466	2.563052
13	D-P-R-G-H-T-G-T-T-N-Q-R-S-P-P	0.939034	14.91164	0.575891	2.592224
14	H-T-G-T-T-N-Q-R-S-P-P-G-E-R-T	1.573387	11.88645	0.981769	1.791049
15	T-N-Q-R-S-P-P-G-E-R-T-R-G-R-Q	7.278548	18.35817	1.629961	3.709075
16	S-P-P-G-E-R-T-R-G-R-Q-P-G-D-Y	69.67598	20.86103	11.47703	4.894688
17	E-R-T-R-G-R-Q-P-G-D-Y-D-D-D-R	90.99506	26.06869	12.11311	4.538379
18	G-R-Q-P-G-D-Y-D-D-D-R-R-Q-P-R	61.25073	12.83401	2.011607	5.382268
19	G-D-Y-D-D-D-R-R-Q-P-R-R-E-E-G	5.760919	33.8763	1.224083	3.753874
20	D-D-R-R-Q-P-R-R-E-E-G-G-R-W-G	9.615215	33.33456	0.966624	3.652816
21	Q-P-R-R-E-E-G-G-R-W-G-P-A-G-P	15.21398	40.74995	2.387195	5.582301
22	E-E-G-G-R-W-G-P-A-G-P-R-E-R-E	11.97396	28.45117	1.77535	3.637188
23	R-W-G-P-A-G-P-R-E-R-E-R-E-E-D	3.990352	26.67645	0.80609	3.573636
24	A-G-P-R-E-R-E-R-E-E-D-W-R-Q-P	4.797345	42.96252	2.072186	6.035501
25	E-R-E-R-E-E-D-W-R-Q-P-R-E-D-W	3.633026	29.95599	2.753696	5.488536
26	E-E-D-W-R-Q-P-R-E-D-W-R-R-P-S	7.629851	28.00263	3.874403	6.090719
27	R-Q-P-R-E-D-W-R-R-P-S-H-Q-Q-P	11.23924	34.16756	4.374177	9.142265
28	E-D-W-R-R-P-S-H-Q-Q-P-R-K-I-R	22.73187	33.66271	3.695696	6.788751
29	R-P-S-H-Q-Q-P-R-K-I-R-P-E-G-R	39.80118	32.43167	4.240904	5.548963
30	Q-Q-P-R-K-I-R-P-E-G-R-E-G-E-Q	13.61605	36.15101	3.041445	4.726952
31	K-I-R-P-E-G-R-E-G-E-Q-E-W-G-T	8.03937	34.48017	1.218026	5.280168
32	E-G-R-E-G-E-Q-E-W-G-T-P-G-S-H	4.843516	25.42016	0.83638	3.067302
33	G-E-Q-E-W-G-T-P-G-S-H-V-R-E-E	3.450349	26.70169	1.420964	4.702989
34	W-G-T-P-G-S-H-V-R-E-E-T-S-R-N	2.723653	26.77159	0.569833	3.004792
35	G-S-H-V-R-E-E-T-S-R-N-N-P-F-Y	0.896878	18.64748	0.369923	4.644646
36	R-E-E-T-S-R-N-N-P-F-Y-F-P-S-R	3.741428	30.16958	2.308443	7.80246
37	S-R-N-N-P-F-Y-F-P-S-R-R-F-S-T	3.719346	30.28705	2.605278	7.304461
38	P-F-Y-F-P-S-R-R-F-S-T-R-Y-G-N	2.155546	37.53157	1.854102	8.224405

39	P-S-R-R-F-S-T-R-Y-G-N-Q-N-G-R	3.01674	27.7871	2.305414	7.839966
40	F-S-T-R-Y-G-N-Q-N-G-R-I-R-V-L	5.353407	34.82482	2.617394	11.68227
41	Y-G-N-Q-N-G-R-I-R-V-L-Q-R-F-D	4.644779	31.5744	2.047954	8.143142
42	N-G-R-I-R-V-L-Q-R-F-D-Q-R-S-R	2.998673	16.42034	1.929826	4.236245
43	R-V-L-Q-R-F-D-Q-R-S-R-Q-F-Q-N	5.301214	44.58288	1.296778	6.030292
44	R-F-D-Q-R-S-R-Q-F-Q-N-L-Q-N-H	11.44801	50.66528	1.230141	7.441984
45	R-S-R-Q-F-Q-N-L-Q-N-H-R-I-V-Q	4.080687	59.62141	1.678424	12.90226
46	F-Q-N-L-Q-N-H-R-I-V-Q-I-E-A-K	3.241574	61.86894	1.554238	4.495663
47	Q-N-H-R-I-V-Q-I-E-A-K-P-N-T-L	3.064919	39.12668	1.009029	8.996408
48	I-V-Q-I-E-A-K-P-N-T-L-V-L-P-K	7.368883	53.94483	2.414455	8.162937
49	E-A-K-P-N-T-L-V-L-P-K-H-A-D-A	10.11908	36.75391	2.393253	7.493034
50	N-T-L-V-L-P-K-H-A-D-A-D-N-I-L	4.729092	39.96161	2.102475	11.7927
51	L-P-K-H-A-D-A-D-N-I-L-V-I-Q-Q	5.110506	47.58573	2.166083	9.502741
52	A-D-A-D-N-I-L-V-I-Q-Q-G-Q-A-T	4.185074	48.48668	2.920288	15.91734
53	N-I-L-V-I-Q-Q-G-Q-A-T-V-T-V-A	3.594885	50.24199	2.069157	10.09138
54	I-Q-Q-G-Q-A-T-V-T-V-A-N-G-N-N	3.141202	60.37965	1.772321	17.75411
55	Q-A-T-V-T-V-A-N-G-N-N-R-K-S-F	3.484475	68.75909	2.705233	18.54486
56	T-V-A-N-G-N-N-R-K-S-F-N-L-D-E	5.285154	47.24011	2.920288	9.930937
57	G-N-N-R-K-S-F-N-L-D-E-G-H-A-L	4.072657	66.40768	2.590134	17.93955
58	K-S-F-N-L-D-E-G-H-A-L-R-I-P-S	3.20544	38.01602	1.848044	10.13722
59	L-D-E-G-H-A-L-R-I-P-S-G-F-I-S	0.740297	21.51344	0.615267	8.335882
60	H-A-L-R-I-P-S-G-F-I-S-Y-I-L-N	2.761795	30.74821	2.108533	14.3077
61	I-P-S-G-F-I-S-Y-I-L-N-R-H-D-N	2.085286	29.80454	2.668886	9.028705
62	F-I-S-Y-I-L-N-R-H-D-N-Q-N-L-R	2.087293	38.44611	1.91771	12.9033
63	I-L-N-R-H-D-N-Q-N-L-R-V-A-K-I	3.859868	34.21707	3.032359	16.25282
64	H-D-N-Q-N-L-R-V-A-K-I-S-M-P-V	2.797929	40.30433	1.969202	14.16705
65	N-L-R-V-A-K-I-S-M-P-V-N-T-P-G	1.950787	26.37354	1.996462	5.267666
66	A-K-I-S-M-P-V-N-T-P-G-Q-F-E-D	2.326179	32.81321	2.450803	3.161068
67	M-P-V-N-T-P-G-Q-F-E-D-F-F-P-A	4.446042	60.45537	2.553787	17.99008
68	T-P-G-Q-F-E-D-F-F-P-A-S-S-R-D	10.91604	70.16294	2.478063	20.72387
69	F-E-D-F-F-P-A-S-S-R-D-Q-S-S-Y	6.475569	82.78211	2.917259	25.04594
70	F-P-A-S-S-R-D-Q-S-S-Y-L-Q-G-F	7.714163	55.39819	3.156545	8.778663
71	S-R-D-Q-S-S-Y-L-Q-G-F-S-R-N-T	14.46922	48.81095	2.138822	11.41347
72	S-S-Y-L-Q-G-F-S-R-N-T-L-E-A-A	40.57806	53.58367	2.272095	8.23274
73	Q-G-F-S-R-N-T-L-E-A-A-F-N-A-E	9.761758	37.38691	3.032359	8.032707
74	R-N-T-L-E-A-A-F-N-A-E-F-N-E-I	5.809097	43.06058	2.97178	11.48953
75	E-A-A-F-N-A-E-F-N-E-I-R-R-V-L	22.75997	52.10117	52.19503	13.47111
76	N-A-E-F-N-E-I-R-R-V-L-L-E-E-N	3.436297	45.73917	3.459439	8.626555
77	N-E-I-R-R-V-L-L-E-E-N-A-G-G-E	2.438596	44.9586	1.420964	12.88663
78	R-V-L-L-E-E-N-A-G-G-E-Q-E-E-R	2.834063	45.45179	1.947999	11.85417
79	E-E-N-A-G-G-E-Q-E-E-R-G-Q-R-R	6.19252	54.52248	9.126579	14.15455
80	G-G-E-Q-E-E-R-G-Q-R-R-W-S-T-R	4.96597	46.0945	2.705233	11.57183
81	E-E-R-G-Q-R-R-W-S-T-R-S-S-E-N	3.544699	58.4127	1.881363	9.089131
82	Q-R-R-W-S-T-R-S-S-E-N-N-E-G-V	2.906331	44.60521	1.445196	7.662854
83	S-T-R-S-S-E-N-N-E-G-V-I-V-K-V	0.991228	13.22429	0.006451	1.822305

84	S-E-N-N-E-G-V-I-V-K-V-S-K-E-H	0.585724	16.3252	0.981769	3.416318
85	E-G-V-I-V-K-V-S-K-E-H-V-E-E-L	2.498819	28.42398	2.081272	6.987742
86	V-K-V-S-K-E-H-V-E-E-L-T-K-H-A	60.2972	23.90174	1.557266	9.595465
87	K-E-H-V-E-E-L-T-K-H-A-K-S-V-S	2.534953	28.83951	1.708713	8.038958
88	E-E-L-T-K-H-A-K-S-V-S-K-K-G-S	3.847823	43.12757	3.311021	13.67114
89	K-H-A-K-S-V-S-K-K-G-S-E-E-E-G	2.013017	31.41907	1.754147	8.847425
90	S-V-S-K-K-G-S-E-E-E-G-D-I-T-N	2.442611	23.27263	1.884392	8.42548
91	K-G-S-E-E-E-G-D-I-T-N-P-I-N-L	15.26417	53.5293	3.1414	7.682649
92	E-E-G-D-I-T-N-P-I-N-L-R-E-G-E	20.33297	52.64388	5.264685	11.5187
93	I-T-N-P-I-N-L-R-E-G-E-P-D-L-S	5.907462	43.25863	2.038867	8.325463
94	I-N-L-R-E-G-E-P-D-L-S-N-N-F-G	4.014441	67.38824	2.750667	12.95019
95	E-G-E-P-D-L-S-N-N-F-G-K-L-F-E	3.77957	30.50938	1.184707	5.800046
96	D-L-S-N-N-F-G-K-L-F-E-V-K-P-D	9.223763	42.11885	5.061746	8.6974
97	N-F-G-K-L-F-E-V-K-P-D-K-K-N-P	62.96108	36.97527	49.20547	8.225447
98	L-F-E-V-K-P-D-K-K-N-P-Q-L-Q-D	5.829172	40.54218	2.438687	9.619427
99	K-P-D-K-K-N-P-Q-L-Q-D-L-D-M-M	3.293768	44.71492	2.175169	8.276497
100	K-N-P-Q-L-Q-D-L-D-M-M-L-T-C-V	6.612076	41.71498	2.414455	8.607802
101	L-Q-D-L-D-M-M-L-T-C-V-E-I-K-E	3.645071	42.80136	1.851073	8.528622
102	D-M-M-L-T-C-V-E-I-K-E-G-A-L-M	4.468124	31.77537	1.896507	6.232409
103	T-C-V-E-I-K-E-G-A-L-M-L-P-H-F	1.764094	23.70952	0.727338	4.457115
104	I-K-E-G-A-L-M-L-P-H-F-N-S-K-A	3.528639	40.1752	1.857131	8.824504
105	A-L-M-L-P-H-F-N-S-K-A-M-V-I-V	3.482468	24.14834	1.260431	4.309174
106	P-H-F-N-S-K-A-M-V-I-V-V-V-N-K	2.587147	25.85122	1.039318	3.880978
107	S-K-A-M-V-I-V-V-V-N-K-G-T-G-N	1.689819	11.05152	0.024625	1.803551
108	V-I-V-V-V-N-K-G-T-G-N-L-E-L-V	2.424544	21.60665	1.505775	5.404147
109	V-N-K-G-T-G-N-L-E-L-V-A-V-R-K	2.119412	24.70561	1.611787	7.822255
110	T-G-N-L-E-L-V-A-V-R-K-E-Q-Q-Q	4.652809	31.23558	2.123678	7.431566
111	E-L-V-A-V-R-K-E-Q-Q-Q-R-G-R-R	3.974292	34.31707	2.468976	13.53987
112	V-R-K-E-Q-Q-Q-R-G-R-R-E-E-E-E	3.396148	29.36377	2.68706	7.476365
113	Q-Q-Q-R-G-R-R-E-E-E-E-D-E-D-E	2.488782	25.05124	2.484121	9.695482
114	G-R-R-E-E-E-E-D-E-D-E-E-E-E-G	1.679781	22.40663	2.335703	5.356222
115	E-E-E-D-E-D-E-E-E-E-G-S-N-R-E	4.670876	58.0962	2.45989	4.14248
116	E-D-E-E-E-E-G-S-N-R-E-V-R-R-Y	7.172153	60.87575	3.117169	8.570296
117	E-E-G-S-N-R-E-V-R-R-Y-T-A-R-L	8.173869	67.4795	3.365542	13.25961
118	N-R-E-V-R-R-Y-T-A-R-L-K-E-G-D	6.52977	61.5903	2.132764	12.25736
119	R-R-Y-T-A-R-L-K-E-G-D-V-F-I-M	4.273401	45.4916	1.678424	11.29054
120	A-R-L-K-E-G-D-V-F-I-M-P-A-A-H	19.09839	41.4276	1.417935	8.031665
121	E-G-D-V-F-I-M-P-A-A-H-P-V-A-I	15.73391	34.54813	2.820333	6.184484
122	F-I-M-P-A-A-H-P-V-A-I-N-A-S-S	8.13372	13.26895	1.287691	1.745208
123	A-A-H-P-V-A-I-N-A-S-S-E-L-H-L	10.34994	18.10575	1.381588	2.301551
124	V-A-I-N-A-S-S-E-L-H-L-L-G-F-G	48.96416	38.79465	2.362964	5.432277
125	A-S-S-E-L-H-L-L-G-F-G-I-N-A-E	44.57589	69.1785	2.290269	12.55846
126	L-H-L-L-G-F-G-I-N-A-E-N-N-H-R	4.146932	38.46844	2.063099	9.510034
127	G-F-G-I-N-A-E-N-N-H-R-I-F-L-A	2.629303	48.80707	1.920739	7.930606
128	N-A-E-N-N-H-R-I-F-L-A-G-D-K-D	13.19851	35.38112	3.080822	5.777125

129	N-H-R-I-F-L-A-G-D-K-D-N-V-I-D	5.06835	38.2432	3.556365	6.866889
130	F-L-A-G-D-K-D-N-V-I-D-Q-I-E-K	3.837786	43.46543	1.729916	12.16777
131	D-K-D-N-V-I-D-Q-I-E-K-Q-A-K-D	8.948743	31.83362	1.036289	7.908728
132	V-I-D-Q-I-E-K-Q-A-K-D-L-A-F-P	2.536961	21.63383	1.37553	5.570841
133	I-E-K-Q-A-K-D-L-A-F-P-G-S-G-E	3.400162	18.97563	1.554238	6.372015
134	A-K-D-L-A-F-P-G-S-G-E-Q-V-E-K	41.56371	22.05324	2.40234	7.693067
135	A-F-P-G-S-G-E-Q-V-E-K-L-I-K-N	4.849538	32.88506	2.620423	11.74582
136	S-G-E-Q-V-E-K-L-I-K-N-Q-K-E-S	2.56908	24.6726	2.590134	8.122305
137	V-E-K-L-I-K-N-Q-K-E-S-H-F-V-S	2.205732	24.22795	2.641626	6.721031
138	I-K-N-Q-K-E-S-H-F-V-S-A-R-P-Q	2.205732	23.72699	2.74461	7.035667
139	K-E-S-H-F-V-S-A-R-P-Q-S-Q-S-Q	5.682628	35.31705	1.390675	5.685443
140	F-V-S-A-R-P-Q-S-Q-S-Q-S-P-S-S	8.246137	43.15864	2.06007	7.543042
141	R-P-Q-S-Q-S-Q-S-P-S-S-P-E-K-E	5.69668	84.11218	3.347368	10.34246
142	Q-S-Q-S-P-S-S-P-E-K-E-S-P-E-K	6.754604	51.32352	1.293749	10.29246
143	P-S-S-P-E-K-E-S-P-E-K-E-D-Q-E	3.853845	41.56741	1.790495	10.62793
144	E-K-E-S-P-E-K-E-D-Q-E-E-E-N-Q	2.713616	37.02187	1.314952	7.271122
145	P-E-K-E-D-Q-E-E-E-N-Q-G-G-K-G	1.886548	29.07446	1.069608	4.984286
146	D-Q-E-E-E-N-Q-G-G-K-G-P-L-L-S	4.438012	30.88607	1.551209	8.704693
147	E-N-Q-G-G-K-G-P-L-L-S-I-L-K-A	7.499367	47.12458	2.114591	14.44939
148	Q-G-G-K-G-P-L-L-S-I-L-K-A-F-N	4.815412	41.814	2.041896	9.341256
	m (blanks)	10717.22	8541.352	19300.87	11504.88
	s (blanks)	498.1455	515.0104	330.1489	959.8412

7.1.5.2 Calculated Z-scores of Ara h 2 peptides

Table A8: Calculated Z-scores of Ara h 2.01 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each peanut-allergic patient (patients 1-3) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	1	1	2	2	3	3
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-15.6093	-15.6093	-15.837	-15.837	-16.689	-16.689
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	24.88522	24.88522	-5.86766	-5.86766	59.0829	59.0829
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-4.87386	-4.87386	-6.21678	-6.21678	-6.30131	-6.30131
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-7.00265	-7.00265	-7.65226	-7.65226	-7.58173	-7.58173
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-5.60232	-5.60232	-6.02318	-6.02318	-6.28988	-6.28988
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-5.24843	-5.24843	-5.48072	-5.48072	-6.5791	-6.5791
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-7.85529	-7.85529	-8.39668	-8.39668	-8.74548	-8.74548
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	-4.56223	-4.56223	-5.23503	-5.23503	-5.95913	-5.95913
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	-6.50438	50.45201	-7.37746	-8.63089	-6.59976	101.0125
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	-2.6656	67.35533	-4.75173	-7.3996	-4.14034	104.0379
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	10.52902	78.46497	-5.61295	-6.20157	-5.15643	105.189
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	24.89748	57.4557	-3.92433	-5.90315	-1.09104	66.18312
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	2.063132	69.57115	-5.03383	-5.31909	-3.06448	2.182899
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-6.5968	-2.46917	-8.52696	-5.31568	-7.2064	-4.87626
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-3.9879	-3.9879	-3.46818	-3.46818	-3.88176	-3.88176
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-6.72463	-6.72463	-6.81773	-6.81773	-6.73745	-6.73745
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-6.71527	-6.71527	-6.03765	-6.03765	-6.17534	-6.17534
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-3.90081	-3.90081	-3.53767	-3.53767	-3.70113	-3.70113
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-5.75533	-5.75533	-5.71415	-5.71415	-5.36392	-5.36392
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-3.08554	-3.08554	-2.63815	-2.63815	-3.23009	-3.23009
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-3.62827	-3.62827	-3.14614	-3.14614	-3.75113	-3.75113
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-2.80749	-2.80749	-2.71651	-2.71651	-3.13417	-3.13417
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-2.50519	-2.50519	-2.38013	-2.38013	-2.5867	-2.5867
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-1.95556	-1.95556	-2.7433	-2.7433	-3.26308	-3.26308
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-8.4171	-8.4171	-9.06065	-9.06065	-10.0842	-10.0842
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-6.36362	-6.36362	-7.90793	-7.90793	-8.20809	-8.20809
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-6.04957	-6.04957	-6.81515	-6.81515	-7.38515	-7.38515
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-10.0542	-10.0542	-10.4244	-10.4244	-10.7791	-10.7791
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-8.33437	-8.33437	-8.7882	-8.7882	-8.99561	-8.99561
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	-6.11608	-6.11608	-6.06069	-6.06069	-6.95578	-6.95578
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	-7.77094	-7.77094	-7.88059	-7.88059	-8.11028	-8.11028
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	-5.04439	-5.04439	-5.53251	-5.53251	-5.79355	-5.79355
	m (blanks)	9592.208333		10493.32271		8641.234136	
	s (blanks)	379.4304356		413.3282315		469.0740411	

Table A9: Calculated Z-scores of Ara h 2.01 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each peanut-allergic patient (patients 4-6) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	4	4	5	5	6	6
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-16.034	-16.034	-17.0706	-17.0706	-15.8523	-15.8523
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-7.44156	-7.44156	-5.58058	-5.58058	51.91572	51.91572
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-6.27436	-6.27436	-7.41301	-7.41301	-2.94865	-2.94865
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-6.96289	-6.96289	-7.92274	-7.92274	-7.97566	-7.97566
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-4.33781	-4.33781	-6.42141	-6.42141	-6.42816	-6.42816
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-4.02306	-4.02306	-6.18127	-6.18127	-6.15366	-6.15366
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-3.79996	-3.79996	-8.71047	-8.71047	-9.03132	-9.03132
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	-4.96707	-4.96707	-5.90369	-5.90369	-2.56747	-2.56747
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	-7.48847	-8.52555	-7.89264	-8.75144	55.90624	56.00539
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	-4.51986	-7.28309	-5.17681	-7.46052	59.01025	57.94627
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	-2.17419	-6.52974	-5.93918	-6.36355	58.36761	58.81588
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	-1.96642	-6.20248	-4.48643	-6.33512	58.98501	57.80829
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	0.831244	-5.17911	-5.25413	-5.21392	-1.06828	44.31241
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-4.77099	-5.16686	-8.96274	-5.1901	-7.61904	-3.80097
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-3.16531	-3.16531	-3.94346	-3.94346	-3.75623	-3.75623
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-4.1665	-4.1665	-7.01191	-7.01191	-6.75581	-6.75581
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-5.94676	-5.94676	-6.29549	-6.29549	-6.8409	-6.8409
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-3.12277	-3.12277	-3.8991	-3.8991	-4.19802	-4.19802
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-1.87359	-1.87359	-6.02505	-6.02505	-6.34557	-6.34557
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-2.47114	-2.47114	-3.03512	-3.03512	-3.817	-3.817
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-3.10827	-3.10827	-3.31852	-3.31852	-4.3667	-4.3667
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-2.73123	-2.73123	-3.07695	-3.07695	-4.17304	-4.17304
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-1.51882	-1.51882	-2.84764	-2.84764	-4.03821	-4.03821
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-2.82996	-2.82996	-3.02953	-3.02953	-4.09812	-4.09812
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-8.7023	-8.7023	-10.1329	-10.1329	-9.64301	-9.64301
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-7.77342	-7.77342	-8.39344	-8.39344	-7.14176	-7.14176
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-6.62425	-6.62425	-7.29984	-7.29984	-6.34687	-6.34687
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-9.94065	-9.94065	-11.2671	-11.2671	-10.8884	-10.8884
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-5.14938	-5.14938	-9.31585	-9.31585	-9.07486	-9.07486
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	-3.84026	-3.84026	-7.39172	-7.39172	-7.49798	-7.49798
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	-7.75516	-7.75516	-8.04786	-8.04786	-8.22812	-8.22812
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	-5.35739	-5.35739	-5.80933	-5.80933	-5.94022	-5.94022
	m (blanks)	9287.655706		10237.31141		10105.10092	
	s (blanks)	467.2674622		444.6433874		799.5560648	

Table A10: Calculated Z-scores of Ara h 2.01 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each peanut-allergic patient (patients 7-9) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	7	7	8	8	9	9
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-14.4476	-14.4476	-15.9077	-15.9077	-17.2683	-17.2683
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	95.69471	95.69471	61.10624	61.10624	-8.33003	-8.33003
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	9.238021	9.238021	-3.8278	-3.8278	-7.47646	-7.47646
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-7.50257	-7.50257	-7.69436	-7.69436	-7.83942	-7.83942
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-6.38187	-6.38187	-6.09855	-6.09855	-6.13145	-6.13145
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-6.31751	-6.31751	-6.3266	-6.3266	-6.1345	-6.1345
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-7.90387	-7.90387	-9.27057	-9.27057	-8.72767	-8.72767
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	-6.2985	-6.2985	-6.10366	-6.10366	-5.60088	-5.60088
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	-7.13438	102.8193	-1.61687	67.89819	-7.64128	-4.41413
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	40.34229	105.6412	21.63283	70.01325	-5.03924	0.681385
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	51.96222	106.1379	13.10152	71.14677	-5.91251	4.091164
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	0.3607	100.4569	44.46546	70.47244	-1.89835	-0.52399
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-1.90809	47.56017	-1.74783	64.89771	-4.53255	0.801029
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-6.98664	-3.24033	-7.31896	-0.98894	-8.33918	-4.18569
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-2.82447	-2.82447	-4.04253	-4.04253	-3.92705	-3.92705
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-6.38536	-6.38536	-7.01856	-7.01856	-6.97447	-6.97447
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-6.44016	-6.44016	-6.72121	-6.72121	-6.48997	-6.48997
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-4.29073	-4.29073	-4.44403	-4.44403	-3.90429	-3.90429
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-6.24939	-6.24939	-6.53757	-6.53757	-5.92693	-5.92693
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-3.41191	-3.41191	-3.88966	-3.88966	-3.19863	-3.19863
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-3.92372	-3.92372	-4.32659	-4.32659	-3.79856	-3.79856
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-3.34883	-3.34883	-3.83497	-3.83497	-3.25885	-3.25885
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-2.84324	-2.84324	-3.80769	-3.80769	-2.73745	-2.73745
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-3.67502	-3.67502	-4.16047	-4.16047	-2.92809	-2.92809
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-8.2292	-8.2292	-9.46967	-9.46967	-9.78381	-9.78381
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-2.63336	-2.63336	-7.51587	-7.51587	-8.26953	-8.26953
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-6.89182	-6.89182	-7.12252	-7.12252	-7.24864	-7.24864
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-10.0466	-10.0466	-10.7588	-10.7588	-10.724	-10.724
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-8.5297	-8.5297	-8.68611	-8.68611	-8.56673	-8.56673
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	-7.00544	-7.00544	-7.43377	-7.43377	-6.96476	-6.96476
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	-8.06217	-8.06217	-8.1199	-8.1199	-8.00979	-8.00979
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	-6.12965	-6.12965	-5.96241	-5.96241	-5.49849	-5.49849
	m (blanks)	6735.093596		10444.14595		8851.646586	
	s (blanks)	479.5367787		668.9231327		450.4718604	

Table A11: Calculated Z-scores of Ara h 2.01 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each peanut-allergic patient (patients 10-12) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	10	10	11	11	12	12
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	34.90244	34.90244	-15.9818	-15.9818	-17.8098	-17.8098
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	77.86174	77.86174	-7.26357	-7.26357	13.83561	13.83561
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	53.44922	53.44922	-5.3545	-5.3545	-3.56168	-3.56168
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	7.806267	7.806267	-6.64606	-6.64606	-8.54491	-8.54491
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	3.38525	3.38525	-5.42017	-5.42017	-5.86026	-5.86026
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	26.37885	26.37885	-4.61825	-4.61825	-6.34004	-6.34004
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	2.068571	2.068571	-7.73704	-7.73704	-8.12188	-8.12188
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	4.426784	4.426784	-4.51894	-4.51894	-4.22012	-4.22012
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	76.40374	79.28816	-7.039	-8.72365	67.74573	69.60111
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	80.25448	81.43362	-4.2905	-7.06556	71.11914	71.83692
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	77.71787	82.48198	-5.50871	-5.85081	68.56887	72.73969
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	81.59492	82.49334	-3.34859	-6.21098	66.0015	71.05328
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	60.24778	82.81043	-2.91201	-4.98538	-2.40095	62.6302
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	29.58067	48.48469	-7.70878	-4.59509	-7.59642	-4.47731
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	3.14076	3.14076	-2.73329	-2.73329	-4.04453	-4.04453
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-2.61696	-2.61696	-5.30176	-5.30176	-7.15365	-7.15365
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-1.25619	-1.25619	-5.6368	-5.6368	-6.99015	-6.99015
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	1.889151	1.889151	-2.5663	-2.5663	-3.97514	-3.97514
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-1.04855	-1.04855	-4.3117	-4.3117	-6.11518	-6.11518
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	7.531052	7.531052	-1.80271	-1.80271	-3.2052	-3.2052
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	2.154222	2.154222	-2.02491	-2.02491	-3.8255	-3.8255
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	5.984522	5.984522	-1.65396	-1.65396	-3.44586	-3.44586
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	15.79713	15.79713	-1.43365	-1.43365	-3.0482	-3.0482
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	65.87531	65.87531	-1.72775	-1.72775	-2.8405	-2.8405
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	77.20752	77.20752	-9.6337	-9.6337	-9.67242	-9.67242
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	78.60053	78.60053	-7.29348	-7.29348	-8.90514	-8.90514
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	22.67847	22.67847	-6.33612	-6.33612	-8.29259	-8.29259
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	5.618181	5.618181	-10.2303	-10.2303	-11.5797	-11.5797
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	8.58615	8.58615	-6.90792	-6.90792	-9.54587	-9.54587
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	-4.46196	-4.46196	-6.06212	-6.06212	-7.93377	-7.93377
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	-2.61169	-2.61169	-7.20712	-7.20712	-8.57672	-8.57672
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	1.861737	1.861737	-4.97037	-4.97037	-6.03808	-6.03808
	m (blanks)	9362.16		8731.827506		10648.40678	
	s (blanks)	586.3116638		301.7226214		649.1258417	

Table A12: Calculated Z-scores of Ara h 2.01 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each peanut-allergic patient (patients 13-15) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	13	13	14	14	15	15
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-14.7109	-14.7109	-14.3288	-14.3288	-17.7549	-17.7549
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-6.53269	-6.53269	-5.55297	-5.55297	-1.04593	-1.04593
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-5.32674	-5.32674	-5.67415	-5.67415	-6.20294	-6.20294
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-6.27821	-6.27821	-5.90228	-5.90228	-6.9653	-6.9653
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-4.49461	-4.49461	-4.52588	-4.52588	-6.44673	-6.44673
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-4.40416	-4.40416	-4.17412	-4.17412	-5.35961	-5.35961
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-7.26123	-7.26123	-6.38768	-6.38768	-9.64886	-9.64886
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	-3.85701	-3.85701	-4.08935	-4.08935	-6.02544	-6.02544
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	-6.49767	-5.12675	-5.89138	-7.45829	46.71625	74.55703
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	-3.82701	-3.84307	-3.73324	-5.6671	64.10364	76.55486
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	-4.46734	-1.04848	-3.78522	-4.36366	57.78697	77.71325
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	-1.35417	-3.26083	-2.0679	-4.56282	50.54214	77.39425
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-2.42721	-3.5721	-2.66344	-3.22683	20.554	77.17393
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-5.97438	-4.0885	-7.10543	-3.92467	6.379057	9.471324
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-1.68813	-1.68813	-1.66895	-1.66895	-1.05645	-1.05645
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-4.68084	-4.68084	-5.00329	-5.00329	-6.15518	-6.15518
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-4.54011	-4.54011	-4.65663	-4.65663	-6.01381	-6.01381
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-1.35293	-1.35293	-1.74551	-1.74551	-2.57572	-2.57572
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-3.17075	-3.17075	-2.8896	-2.8896	-5.67057	-5.67057
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-0.02071	-0.02071	0.275816	0.275816	-3.3901	-3.3901
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-0.47059	-0.47059	-0.77225	-0.77225	-3.96937	-3.96937
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	0.138865	0.138865	-0.45385	-0.45385	-3.6236	-3.6236
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	0.667789	0.667789	0.84444	0.84444	-3.10565	-3.10565
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	0.539898	0.539898	0.61623	0.61623	-3.08725	-3.08725
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-7.87056	-7.87056	-7.8961	-7.8961	10.38215	10.38215
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-6.19471	-6.19471	-6.42128	-6.42128	70.46785	70.46785
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-5.96223	-5.96223	-5.55134	-5.55134	2.545822	2.545822
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-9.09529	-9.09529	-9.14142	-9.14142	-9.87605	-9.87605
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-6.4216	-6.4216	-6.27616	-6.27616	-8.034	-8.034
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	-5.87594	-5.87594	-5.3597	-5.3597	-6.47082	-6.47082
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	-6.79957	-6.79957	-6.00209	-6.00209	-8.56262	-8.56262
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	-4.0438	-4.0438	-3.44199	-3.44199	-6.09044	-6.09044
	m (blanks)	9020.593472		9055.216301		8750.54717	
	s (blanks)	199.1464024		198.9558915		626.7018591	

Table A13: Calculated Z-scores of Ara h 2.01 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each peanut-allergic patient (patients 16-18) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	16	16	17	17	18	18
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-16.5582	-16.5582	-16.3451	-16.3451	-16.0744	-16.0744
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-7.88047	-7.88047	73.6812	73.6812	93.44976	93.44976
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-6.79672	-6.79672	69.92526	69.92526	19.02107	19.02107
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-7.78089	-7.78089	-5.34555	-5.34555	-1.88096	-1.88096
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-5.58071	-5.58071	-6.04793	-6.04793	-1.47517	-1.47517
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-5.87647	-5.87647	-4.98714	-4.98714	3.853184	3.853184
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-7.18918	-7.18918	-9.06053	-9.06053	-6.0447	-6.0447
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	-5.19417	-5.19417	-5.8386	-5.8386	-1.42439	-1.42439
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	-7.19171	5.729859	-7.20573	61.51052	93.08172	102.2842
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	-4.88902	8.039574	-4.21562	72.17792	96.82627	104.4802
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	-5.371	16.41597	-5.83812	69.51945	87.17452	105.6427
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	-2.98534	11.99356	-1.66842	52.48091	105.5101	105.4889
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-3.86616	2.28513	-4.43115	26.62258	88.44766	105.5339
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-7.82748	-4.6469	-8.35633	-3.27074	45.5348	66.87927
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-2.92936	-2.92936	-4.08974	-4.08974	5.658221	5.658221
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-6.17857	-6.17857	-7.13607	-7.13607	-5.22368	-5.22368
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-5.99052	-5.99052	-6.96013	-6.96013	-5.49551	-5.49551
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-2.88884	-2.88884	-3.81331	-3.81331	-1.56216	-1.56216
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-4.64394	-4.64394	-6.1348	-6.1348	-3.53141	-3.53141
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-2.09137	-2.09137	-3.44831	-3.44831	-2.26763	-2.26763
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-2.36326	-2.36326	-3.75596	-3.75596	-2.51895	-2.51895
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-1.99669	-1.99669	-3.23375	-3.23375	-2.46322	-2.46322
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-1.855	-1.855	-2.89908	-2.89908	-0.94734	-0.94734
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-1.90765	-1.90765	-2.81963	-2.81963	3.373468	3.373468
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-9.75742	-9.75742	-9.94956	-9.94956	48.1538	48.1538
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-7.98079	-7.98079	-7.40712	-7.40712	92.42276	92.42276
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-7.03544	-7.03544	-7.38833	-7.38833	2.326035	2.326035
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-10.9152	-10.9152	-11.1958	-11.1958	-6.0797	-6.0797
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-8.06822	-8.06822	-8.76832	-8.76832	-6.26779	-6.26779
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	-6.60362	-6.60362	-7.46755	-7.46755	-5.6742	-5.6742
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	-7.70452	-7.70452	-8.47639	-8.47639	-5.47405	-5.47405
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	-5.48437	-5.48437	-6.01524	-6.01524	-4.64974	-4.64974
	m (blanks)	9243.229314		11596.62551		10027.6129	
	s (blanks)	319.4069177		597.7429055		465.2042138	

Table A14: Calculated Z-scores of Ara h 2.01 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each peanut-allergic patient (patients 19-21) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	19	19	20	20	21	21
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-18.8479	-18.8479	-17.3958	-17.3958	-10.692	-10.692
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-9.49114	-9.49114	-6.4329	-6.4329	19.0295	19.0295
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-8.42145	-8.42145	-6.93501	-6.93501	13.57041	13.57041
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-8.77158	-8.77158	-8.27701	-8.27701	-5.72108	-5.72108
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-6.98684	-6.98684	-6.24875	-6.24875	-1.95257	-1.95257
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-6.83238	-6.83238	-6.1527	-6.1527	-0.91571	-0.91571
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-9.69779	-9.69779	-9.08661	-9.08661	-4.9797	-4.9797
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	-5.97311	-5.97311	-5.89388	-5.89388	10.54227	10.54227
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	-7.88123	-9.80357	-7.62729	55.79632	21.25456	20.12038
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	-4.43948	-5.63887	-5.24122	43.24526	24.02745	21.73475
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	-5.43235	-4.32516	-6.04807	55.90512	23.16422	22.81892
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	-4.82232	-6.77996	-4.02221	8.32792	24.7854	23.02096
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-5.60346	-5.98881	-4.82291	-1.36421	22.88784	23.93745
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-9.11512	-5.9428	-8.38959	-4.50856	9.59778	14.18211
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-3.8364	-3.8364	-3.68774	-3.68774	3.213946	3.213946
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-7.07964	-7.07964	-6.42866	-6.42866	-1.99622	-1.99622
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-6.79922	-6.79922	-6.31247	-6.31247	-2.80329	-2.80329
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-4.0151	-4.0151	-3.59415	-3.59415	-0.28184	-0.28184
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-6.06909	-6.06909	-5.35637	-5.35637	-3.97071	-3.97071
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-3.32256	-3.32256	-2.67321	-2.67321	-2.68056	-2.68056
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-3.91461	-3.91461	-3.04905	-3.04905	-1.86458	-1.86458
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-4.33977	-4.33977	-2.56758	-2.56758	-3.84413	-3.84413
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-4.13791	-4.13791	-2.10077	-2.10077	1.544414	1.544414
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-4.25733	-4.25733	-2.56136	-2.56136	-3.28397	-3.28397
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-11.4855	-11.4855	-9.94244	-9.94244	-2.78567	-2.78567
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-9.66175	-9.66175	-8.53565	-8.53565	-4.83594	-4.83594
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-8.42903	-8.42903	-7.12196	-7.12196	-5.8296	-5.8296
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-11.7138	-11.7138	-10.6796	-10.6796	-8.4868	-8.4868
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-9.51595	-9.51595	-8.86204	-8.86204	-6.96985	-6.96985
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	-7.56632	-7.56632	-7.25394	-7.25394	-6.08432	-6.08432
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	-8.16934	-8.16934	-8.08462	-8.08462	-6.15732	-6.15732
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	-5.90544	-5.90544	-5.76256	-5.76256	0.386153	0.386153
	m (blanks)	26168.36654		8583.473684		10659.28796	
	s (blanks)	9679.687758		342.5203501		1674.184356	

Table A15: Calculated Z-scores of Ara h 2.01 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each peanut-allergic patient (patients 22-23) and peanut-tolerant patient 24 are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	22	22	23	23	24	24
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-16.6399	-16.6399	-15.6171	-15.6171	-15.6128	-15.6128
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-1.27362	-1.27362	62.1056	62.1056	-6.93763	-6.93763
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-6.23693	-6.23693	-3.79314	-3.79314	-6.10302	-6.10302
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-7.25223	-7.25223	-7.62967	-7.62967	-5.98791	-5.98791
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-5.94598	-5.94598	-6.10374	-6.10374	-5.26139	-5.26139
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-5.22242	-5.22242	-5.85019	-5.85019	-4.81836	-4.81836
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-9.05493	-9.05493	-9.1365	-9.1365	-7.30082	-7.30082
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	-4.88343	-4.88343	-4.88203	-4.88203	-4.74749	-4.74749
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	53.57238	78.92373	64.9842	72.68565	-7.09529	-7.82299
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	48.01901	80.7125	75.17871	74.79566	-4.4566	-6.68099
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	21.04359	81.6537	73.82538	75.40046	-4.62491	-5.76982
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	80.54702	81.00486	65.27153	72.35843	-3.28592	-5.28894
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	8.764985	78.6878	2.406967	60.40548	-4.51761	-4.82614
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-5.12402	1.574228	-7.26903	-2.91505	-8.00328	-4.56013
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-3.75026	-3.75026	-3.80419	-3.80419	-3.26279	-3.26279
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-6.92884	-6.92884	-6.88913	-6.88913	-5.90084	-5.90084
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-6.56524	-6.56524	-6.76973	-6.76973	-5.42116	-5.42116
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-3.98639	-3.98639	-4.3532	-4.3532	-3.27003	-3.27003
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-6.14108	-6.14108	-6.44635	-6.44635	-5.6705	-5.6705
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-3.57284	-3.57284	-3.9137	-3.9137	-2.30145	-2.30145
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-3.97929	-3.97929	-4.41712	-4.41712	-3.03544	-3.03544
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-3.83171	-3.83171	-4.11809	-4.11809	-2.44722	-2.44722
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-3.38208	-3.38208	-2.7914	-2.7914	-2.48563	-2.48563
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-3.52935	-3.52935	54.65392	54.65392	-2.59404	-2.59404
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-8.25758	-8.25758	0.726862	0.726862	-7.54432	-7.54432
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-7.0386	-7.0386	-6.25742	-6.25742	-6.91186	-6.91186
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-6.46838	-6.46838	-6.71567	-6.71567	-6.0481	-6.0481
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-10.2871	-10.2871	-10.5324	-10.5324	-9.99401	-9.99401
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-8.18412	-8.18412	-8.81047	-8.81047	-7.39489	-7.39489
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	-6.40301	-6.40301	-7.21732	-7.21732	-5.3693	-5.3693
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	-8.10192	-8.10192	-8.25982	-8.25982	-6.83499	-6.83499
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	-5.91261	-5.91261	-5.79713	-5.79713	-5.30066	-5.30066
	m (blanks)	9950.296073		9928.151057		11096.52677	
	s (blanks)	595.2847421		639.6703802		403.5663398	

Table A16: Calculated Z-scores of Ara h 2.01 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each peanut-tolerant patient (patients 25-27) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	25	25	26	26	27	27
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-14.403	-14.403	-20.1323	-20.1323	-15.8076	-15.8076
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-6.01015	-6.01015	-11.0327	-11.0327	-7.75596	-7.75596
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-4.21737	-4.21737	-9.42864	-9.42864	-6.5184	-6.5184
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-5.8365	-5.8365	-10.5685	-10.5685	-6.50266	-6.50266
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-2.75056	-2.75056	-8.66636	-8.66636	-5.51326	-5.51326
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-3.41084	-3.41084	-8.21585	-8.21585	-4.89704	-4.89704
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-4.66993	-4.66993	-10.4581	-10.4581	-5.48119	-5.48119
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	-3.46197	-3.46197	-7.34271	-7.34271	-4.69147	-4.69147
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	-5.61074	-6.56676	-8.78813	-10.3758	-6.91386	-7.7332
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	-1.91178	-4.82824	-5.29738	-9.04915	-4.54837	-6.64946
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	-3.85716	-3.93796	-6.49369	-8.25358	-5.06727	-5.56856
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	-2.03711	-3.41736	-4.76431	-8.06179	-2.95667	-5.4287
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-0.99995	-2.55159	-4.747	-6.69918	-2.31304	-4.70111
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-5.90191	-2.28449	-8.72375	-6.42734	-5.73057	-4.61289
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-2.67444	-2.67444	-4.25869	-4.25869	-2.96116	-2.96116
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-4.9155	-4.9155	-6.65536	-6.65536	-4.50414	-4.50414
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-6.44484	-6.44484	-6.1806	-6.1806	-5.24058	-5.24058
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-3.0167	-3.0167	-3.12349	-3.12349	-3.09262	-3.09262
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-6.04653	-6.04653	-4.62019	-4.62019	-4.84881	-4.84881
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-3.10656	-3.10656	-1.16117	-1.16117	-2.69461	-2.69461
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-4.14433	-4.14433	-1.99643	-1.99643	-3.23534	-3.23534
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-4.03421	-4.03421	-0.39939	-0.39939	-2.35837	-2.35837
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-3.44764	-3.44764	-0.85233	-0.85233	-2.38317	-2.38317
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-4.00782	-4.00782	-0.56959	-0.56959	-2.73132	-2.73132
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-5.2364	-5.2364	-12.9257	-12.9257	-8.22009	-8.22009
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-4.88895	-4.88895	-11.0496	-11.0496	-6.83468	-6.83468
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-5.22516	-5.22516	-9.88632	-9.88632	-5.72654	-5.72654
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-8.234	-8.234	-13.1876	-13.1876	-9.69944	-9.69944
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-5.45557	-5.45557	-10.8999	-10.8999	-6.14743	-6.14743
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	-3.57322	-3.57322	-8.93191	-8.93191	-5.6722	-5.6722
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	-5.11988	-5.11988	-9.02178	-9.02178	-7.42233	-7.42233
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	-3.00069	-3.00069	-7.18025	-7.18025	-4.93121	-4.93121
	m (blanks)	12197.20253		14751.29909		11851.53209	
	s (blanks)	1714.718816		1597.646395		461.1748893	

Table A17: Calculated Z-scores of Ara h 2.01 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each peanut-tolerant patient (patients 28-30) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	28	28	29	29	30	30
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-16.1315	-16.1315	-16.1435	-16.1435	-15.9711	-15.9711
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-7.62222	-7.62222	-7.85828	-7.85828	-7.33023	-7.33023
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-6.51832	-6.51832	-6.36353	-6.36353	-6.01509	-6.01509
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-7.1998	-7.1998	-6.85042	-6.85042	-7.36758	-7.36758
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-5.88483	-5.88483	-5.49648	-5.49648	-5.17322	-5.17322
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-5.37518	-5.37518	-5.67097	-5.67097	-5.48496	-5.48496
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-7.81996	-7.81996	-8.11885	-8.11885	-7.48527	-7.48527
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	-4.99419	-4.99419	-5.22646	-5.22646	-4.74622	-4.74622
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	-7.32541	-7.93583	-7.24292	-7.51373	-6.93503	-5.36634
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	-4.80348	-6.93444	-3.98552	-6.21494	-4.56318	-5.18641
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	-5.49605	-5.82621	-5.12551	-5.77264	-5.52327	-4.35204
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	-3.54339	-5.87909	-4.12231	-5.25581	-3.85339	-4.54282
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-4.27148	-5.02536	-4.76046	-4.34831	-4.51062	-3.90443
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-8.21094	-4.92154	-8.42008	-4.79801	-8.56386	-3.83761
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-3.66215	-3.66215	-3.48713	-3.48713	-3.19686	-3.19686
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-6.32261	-6.32261	-6.81214	-6.81214	-6.37968	-6.37968
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-5.95125	-5.95125	-6.45309	-6.45309	-6.22553	-6.22553
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-3.14778	-3.14778	-3.77435	-3.77435	-3.46751	-3.46751
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-4.51958	-4.51958	-5.66493	-5.66493	-5.56529	-5.56529
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-2.33378	-2.33378	-2.62856	-2.62856	-2.58738	-2.58738
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-3.03036	-3.03036	-3.31512	-3.31512	-3.03907	-3.03907
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-2.53202	-2.53202	-2.97149	-2.97149	-3.11186	-3.11186
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-2.26669	-2.26669	-2.66838	-2.66838	-2.34768	-2.34768
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-2.94247	-2.94247	-3.2777	-3.2777	-2.67929	-2.67929
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-8.78904	-8.78904	-8.71254	-8.71254	-8.29625	-8.29625
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-7.47421	-7.47421	-7.13505	-7.13505	-6.33661	-6.33661
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-6.09591	-6.09591	-6.60001	-6.60001	-5.74918	-5.74918
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-10.1136	-10.1136	-9.76832	-9.76832	-9.37562	-9.37562
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-8.27336	-8.27336	-8.33809	-8.33809	-6.6277	-6.6277
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	-6.45465	-6.45465	-6.74533	-6.74533	-5.55567	-5.55567
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	-7.18427	-7.18427	-7.59955	-7.59955	-7.0514	-7.0514
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	-5.35702	-5.35702	-5.25459	-5.25459	-4.9214	-4.9214
	m (blanks)	12315.06472		14543.81696		10913.91392	
	s (blanks)	500.0454477		384.1710475		327.9266085	

Table A18: Calculated Z-scores of Ara h 2.01 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each peanut-tolerant patient (patients 31-33) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	31	31	32	32	33	33
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-16.7887	-16.7887	-17.6274	-17.6274	-17.2259	-17.2259
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-8.26537	-8.26537	-9.33768	-9.33768	-8.87163	-8.87163
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-7.21115	-7.21115	-7.48401	-7.48401	-7.74605	-7.74605
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-7.50519	-7.50519	-8.15567	-8.15567	-8.1327	-8.1327
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-5.76727	-5.76727	-6.25402	-6.25402	-6.31786	-6.31786
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-5.36563	-5.36563	-6.01389	-6.01389	-6.26277	-6.26277
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-7.98976	-7.98976	-8.45314	-8.45314	-8.70007	-8.70007
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	-5.4683	-5.4683	-5.53614	-5.53614	-5.51828	-5.51828
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	-7.18293	-8.48354	-7.61505	-8.71449	-7.42921	-8.39349
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	-4.61552	-7.16751	-4.96669	-7.48204	-4.78481	-7.39734
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	-5.63514	-6.45595	-5.74031	-6.5245	-5.69911	-6.55528
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	-3.35667	-6.27173	-4.04467	-6.34089	-4.051	-6.00386
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-4.28348	-5.14944	-4.7494	-5.12299	-4.74462	-4.93864
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-7.46789	-5.10172	-8.23087	-5.31731	-8.20714	-5.23644
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-3.30554	-3.30554	-3.56242	-3.56242	-3.44765	-3.44765
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-6.22196	-6.22196	-6.68035	-6.68035	-6.32937	-6.32937
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-6.01436	-6.01436	-6.0449	-6.0449	-6.11147	-6.11147
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-2.99621	-2.99621	-3.52031	-3.52031	-3.64677	-3.64677
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-4.84845	-4.84845	-5.27068	-5.27068	-5.33036	-5.33036
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-2.33978	-2.33978	-2.60911	-2.60911	-2.71434	-2.71434
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-2.46843	-2.46843	-3.07917	-3.07917	-2.84428	-2.84428
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-2.30882	-2.30882	-2.66893	-2.66893	-2.48194	-2.48194
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-1.56976	-1.56976	-2.20574	-2.20574	-2.23401	-2.23401
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-1.58082	-1.58082	-2.62377	-2.62377	-2.60475	-2.60475
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-9.70918	-9.70918	-10.1905	-10.1905	-10.0671	-10.0671
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-8.15775	-8.15775	-8.71408	-8.71408	-8.68301	-8.68301
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-7.50076	-7.50076	-7.40233	-7.40233	-7.62749	-7.62749
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-10.8529	-10.8529	-10.8681	-10.8681	-10.9929	-10.9929
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-8.66204	-8.66204	-8.77289	-8.77289	-8.81125	-8.81125
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	-6.55204	-6.55204	-6.91173	-6.91173	-7.35723	-7.35723
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	-7.56077	-7.56077	-7.97043	-7.97043	-7.98609	-7.98609
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	-5.66254	-5.66254	-5.3136	-5.3136	-5.56505	-5.56505
	m (blanks)	12100.85777		11909.87841		10981.51739	
	s (blanks)	429.7547642		444.6552176		408.3888554	

Table A19: Calculated Z-scores of Ara h 2.01 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each peanut-tolerant patient (patients 34-35) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	34	34	35	35
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-17.8392	-17.8392	-17.6687	-17.6687
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-9.23519	-9.23519	-9.06935	-9.06935
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-7.68299	-7.68299	-7.94408	-7.94408
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-8.78861	-8.78861	-8.12819	-8.12819
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-6.77961	-6.77961	-6.44503	-6.44503
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-7.07231	-7.07231	-6.37625	-6.37625
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-9.6506	-9.6506	-9.02647	-9.02647
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	-6.279	-6.279	-5.90881	-5.90881
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	-8.02753	-9.26037	-7.73411	-8.53431
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	-5.26101	-7.79732	-5.36161	-7.0576
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	-5.98613	-6.70337	-6.05308	-6.43796
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	-4.12088	-6.36759	-4.58381	-5.65342
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-3.89956	-4.71507	-5.56081	-4.73287
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-7.72748	-5.63145	-8.82928	-4.91599
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-3.83128	-3.83128	-4.13538	-4.13538
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-6.68164	-6.68164	-6.94447	-6.94447
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-6.77929	-6.77929	-6.5081	-6.5081
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-4.40655	-4.40655	-4.12249	-4.12249
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-6.03547	-6.03547	-5.79592	-5.79592
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-2.6388	-2.6388	-3.05105	-3.05105
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-3.72641	-3.72641	-3.42906	-3.42906
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-3.69882	-3.69882	-2.84092	-2.84092
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-3.35388	-3.35388	-2.8154	-2.8154
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-3.81816	-3.81816	-3.23611	-3.23611
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-10.5205	-10.5205	-10.2296	-10.2296
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-8.51213	-8.51213	-8.55121	-8.55121
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-7.67201	-7.67201	-7.68731	-7.68731
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-11.328	-11.328	-10.7786	-10.7786
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-9.15491	-9.15491	-9.04834	-9.04834
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	-7.74015	-7.74015	-7.61202	-7.61202
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	-8.74966	-8.74966	-8.06676	-8.06676
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	-6.20049	-6.20049	-5.77861	-5.77861
	m (blanks)	12530.63095		10368.17722	
	s (blanks)	506.3960279		455.1615403	

Table A20: Calculated Z-scores of Ara h 2.01 peptides of controls.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each control are listed. Numbers written behind control sera represent the respective developed X-ray film. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Control No. →	N_2	N_2	N_3	N_3	N_4	N_4
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	2.618215	2.618215	2.289753	2.289753	3.022174	3.022174
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	2.46384	2.46384	1.745793	1.745793	2.937296	2.937296
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	2.178675	2.178675	1.569007	1.569007	2.746965	2.746965
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	2.208692	2.208692	1.617963	1.617963	2.394596	2.394596
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	2.324473	2.324473	1.66148	1.66148	2.152824	2.152824
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	2.315897	2.315897	0.899936	0.899936	1.671852	1.671852
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	2.789743	2.789743	1.481973	1.481973	1.952205	1.952205
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	1.342476	1.342476	1.264389	1.264389	1.406932	1.406932
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	1.160228	0.673518	0.774826	1.074003	1.507242	2.309719
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	1.245992	0.493413	0.758507	0.872738	1.352919	2.085951
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	1.16666	0.221113	0.695952	0.44573	0.959397	2.178544
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	1.676956	0.077458	0.709551	0.598039	1.383784	1.846751
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	2.255862	0.257563	2.390385	0.200948	1.455801	1.468661
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	2.045741	0.017424	2.512776	-0.07647	1.257754	1.059707
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	1.170949	1.170949	1.174636	1.174636	0.671329	0.671329
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	1.597624	1.597624	1.131119	1.131119	0.535011	0.535011
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	1.040159	1.040159	0.826502	0.826502	0.411553	0.411553
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	1.014429	1.014429	0.98969	0.98969	0.645608	0.645608
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	0.920089	0.920089	0.85098	0.85098	0.691905	0.691905
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	0.514854	0.514854	1.579886	1.579886	0.625032	0.625032
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	0.825749	0.825749	0.823782	0.823782	0.694477	0.694477
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	0.881495	0.881495	1.234472	1.234472	0.828223	0.828223
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	1.32318	1.32318	2.053131	2.053131	0.398692	0.398692
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	0.892216	0.892216	1.688678	1.688678	0.645608	0.645608
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	1.434673	1.434673	2.009614	2.009614	2.422888	2.422888
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	1.546166	1.546166	1.949778	1.949778	2.574639	2.574639
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	1.514004	1.514004	2.080329	2.080329	2.129676	2.129676
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	1.218119	1.218119	1.626122	1.626122	2.157968	2.157968
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	2.009291	2.009291	1.751233	1.751233	1.988213	1.988213
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	1.177381	1.177381	1.990575	1.990575	1.710433	1.710433
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	0.838613	0.838613	1.25079	1.25079	1.085427	1.085427
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	0.757138	0.757138	0.627957	0.627957	1.093143	1.093143
	m (blanks)	9170.873662		8143.116379		11455.98985	
	s (blanks)	466.3965312		367.6744314		388.7963548	

Table A21: Calculated Z-scores of Ara h 2.01 peptides of controls.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each control are listed. Numbers written behind control sera represent the respective developed X-ray film. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Control No. →	DLab71S1_1	DLab71S1_1	DLab71S1_2	DLab71S1_2	DLab71S1_3	DLab71S1_3
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	2.490168	2.490168	2.887099	2.887099	2.550297	2.550297
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	1.48453	1.48453	2.551398	2.551398	2.118537	2.118537
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	1.005075	1.005075	2.418741	2.418741	2.128406	2.128406
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	0.633293	0.633293	2.080332	2.080332	0.976222	0.976222
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	1.04977	1.04977	1.768996	1.768996	1.193336	1.193336
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	1.02539	1.02539	1.590316	1.590316	0.976222	0.976222
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	1.482499	1.482499	1.936847	1.936847	1.000894	1.000894
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	0.651577	0.651577	1.48744	1.48744	0.865198	0.865198
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	0.519524	0.296049	1.092178	1.944969	0.433437	1.662104
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	0.466703	0.151806	1.046154	1.552414	1.220475	1.213073
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	1.021327	-0.14074	1.855629	1.254614	0.971287	0.773911
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	0.720652	-0.26467	1.509098	1.503683	1.220475	0.875066
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	1.48453	-0.09808	1.9937	1.495562	1.588088	0.512388
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	1.370761	0.076637	1.766289	0.518236	1.889087	0.396429
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	0.446387	0.446387	1.113836	1.113836	1.15386	1.15386
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	0.657672	0.657672	0.918913	0.918913	0.618477	0.618477
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	0.99898	0.99898	0.680673	0.680673	0.376691	0.376691
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	1.027422	1.027422	0.518236	0.518236	0.603674	0.603674
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	1.204171	1.204171	1.113836	1.113836	1.158795	1.158795
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	1.393109	1.393109	0.761891	0.761891	0.640682	0.640682
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	0.812073	0.812073	0.242095	0.242095	0.455642	0.455642
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	1.722227	1.722227	1.062398	1.062398	0.645616	0.645616
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	1.447962	1.447962	1.084056	1.084056	1.225409	1.225409
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	1.571889	1.571889	1.154445	1.154445	0.949082	0.949082
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	1.244802	1.244802	3.017048	3.017048	2.261635	2.261635
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	1.261055	1.261055	2.602836	2.602836	2.345519	2.345519
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	0.8974	0.8974	2.17238	2.17238	1.935964	1.935964
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	0.517492	0.517492	1.741923	1.741923	1.437589	1.437589
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	1.324034	1.324034	2.296914	2.296914	2.505888	2.505888
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	1.12697	1.12697	1.750045	1.750045	1.42772	1.42772
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	0.25948	0.25948	0.983887	0.983887	0.608608	0.608608
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	0.547966	0.547966	1.13008	1.13008	0.791181	0.791181
	m (blanks)	11243.27745		11721.57619		9079.320513	
	s (blanks)	492.2247585		369.3755052		405.3172388	

Table A22: Calculated Z-scores of Ara h 2.01 peptides of controls.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each control are listed. Numbers written behind control sera represent the respective developed X-ray film. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Control No. →	DLab71S1_4	DLab71S1_4	DLab72S1_1	DLab72S1_1	DLab72S1_2	DLab72S1_2
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	1.554046	1.554046	1.91215	1.91215	2.307903	2.307903
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	1.661996	1.661996	0.798671	0.798671	1.742894	1.742894
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	2.303697	2.303697	1.347073	1.347073	1.591359	1.591359
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	1.627512	1.627512	0.704182	0.704182	1.420341	1.420341
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	1.137241	1.137241	0.66157	0.66157	0.82719	0.82719
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	0.828385	0.828385	0.291028	0.291028	1.032844	1.032844
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	2.723501	2.723501	1.085841	1.085841	1.097788	1.097788
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	1.795434	1.795434	0.278059	0.278059	0.604217	0.604217
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	0.675457	1.269179	0.226183	0.48371	0.675655	1.708257
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	1.212206	1.104256	0.580051	0.376253	0.51979	1.42467
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	0.985812	0.829884	0.59302	0.100199	0.61937	0.944088
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	0.564508	0.886858	0.685655	-0.0369	1.242828	0.853167
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	1.042785	0.834382	1.185887	-0.04431	0.92677	0.699467
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	0.141706	0.669459	0.596725	-0.14065	0.935429	0.47
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	0.41158	0.41158	0.291028	0.291028	0.6237	0.6237
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	0.254153	0.254153	0.498531	0.498531	0.686479	0.686479
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	3.705542	3.705542	0.433687	0.433687	0.335783	0.335783
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-0.00523	-0.00523	0.828314	0.828314	0.513296	0.513296
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	0.551015	0.551015	0.909833	0.909833	0.539273	0.539273
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	0.318623	0.318623	1.002469	1.002469	0.396397	0.396397
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	0.048749	0.048749	0.568934	0.568934	0.580404	0.580404
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	0.416078	0.416078	1.076577	1.076577	0.467835	0.467835
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	0.387591	0.387591	1.178476	1.178476	0.359596	0.359596
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	0.210673	0.210673	1.302608	1.302608	0.664831	0.664831
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	3.834482	3.834482	1.636096	1.636096	1.745059	1.745059
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	1.576536	1.576536	1.967731	1.967731	1.548063	1.548063
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	2.140273	2.140273	0.991352	0.991352	1.257982	1.257982
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	0.912346	0.912346	0.454066	0.454066	1.766706	1.766706
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	1.52556	1.52556	1.247026	1.247026	1.322925	1.322925
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	2.257218	2.257218	0.657865	0.657865	0.863991	0.863991
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	0.726433	0.726433	0.331787	0.331787	0.894298	0.894298
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	0.756419	0.756419	0.194687	0.194687	0.565251	0.565251
	m (blanks)	11903.48548		11209.91781		11229.88839	
	s (blanks)	666.977806		539.7497324		461.9395702	

Table A23: Calculated Z-scores of Ara h 2.01 peptides of controls and derived maximum Z-score.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp for each control and the maximum Z-score (Max.) of the controls are listed. Numbers written behind control sera represent the respective developed X-ray film. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Control No. →	DLab72S1_3	DLab72S1_3	DLab72S1_4	DLab72S1_4	Max.	Max.
	Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	2.898064	2.898064	18.05717	18.05717	18.05717	18.05717
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	2.144974	2.144974	9.391927	9.391927	9.391927	9.391927
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	2.429927	2.429927	7.970057	7.970057	7.970057	7.970057
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	1.663268	1.663268	8.459553	8.459553	8.459553	8.459553
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	1.699452	1.699452	6.600633	6.600633	6.600633	6.600633
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	0.926009	0.926009	6.45495	6.45495	6.45495	6.45495
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	1.84419	1.84419	9.467682	9.467682	9.467682	9.467682
8	M-Q-K-I-Q-R-D-E-D-S-Y-E-R-D-P	0.833286	0.833286	5.947972	5.947972	5.947972	5.947972
9	Q-R-D-E-D-S-Y-E-R-D-P-Y-S-**P**-S	0.394549	1.082055	7.795237	9.065596	7.795237	9.065596
10	D-S-Y-E-R-D-P-Y-S-**P**-S-Q-D-P-Y	0.650102	0.876255	5.29531	7.626244	5.29531	7.626244
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-P	0.711164	0.846855	6.221856	6.554015	6.221856	6.554015
12	S-**P**-S-Q-D-P-Y-S-**P**-S-P-Y-D-R-R	0.885302	0.324442	4.625166	6.320921	4.625166	6.320921
13	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	1.789914	0.575472	5.703224	5.231209	5.703224	5.231209
14	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	1.165731	0.168396	9.094733	5.225382	9.094733	5.225382
15	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	0.663672	0.663672	3.989987	3.989987	3.989987	3.989987
16	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	0.797102	0.797102	7.148403	7.148403	7.148403	7.148403
17	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	1.127285	1.127285	6.857036	6.857036	6.857036	6.857036
18	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	1.631607	1.631607	4.07157	4.07157	4.07157	4.07157
19	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	0.559641	0.559641	6.10531	6.10531	6.10531	6.10531
20	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	0.299565	0.299565	3.26157	3.26157	3.26157	3.26157
21	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	0.091504	0.091504	3.646174	3.646174	3.646174	3.646174
22	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	0.607133	0.607133	3.5238	3.5238	3.5238	3.5238
23	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	0.844594	0.844594	3.366462	3.366462	3.366462	3.366462
24	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	0.659148	0.659148	3.710275	3.710275	3.710275	3.710275
25	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	2.016067	2.016067	10.71473	10.71473	10.71473	10.71473
26	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	2.158543	2.158543	9.053941	9.053941	9.053941	9.053941
27	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	1.457469	1.457469	7.917611	7.917611	7.917611	7.917611
28	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	2.429927	2.429927	11.43732	11.43732	11.43732	11.43732
29	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	2.090697	2.090697	9.351136	9.351136	9.351136	9.351136
30	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-D-V	0.978024	0.978024	7.620417	7.620417	7.620417	7.620417
31	L-R-A-P-Q-R-C-D-L-D-V-E-S-G-G	0.756394	0.756394	8.308043	8.308043	8.308043	8.308043
32	Q-R-C-D-L-D-V-E-S-G-G-R-D-R-Y	0.704379	0.704379	5.918835	5.918835	5.918835	5.918835
	m (blanks)	8431.538776		10277.29825			
	s (blanks)	442.1784235		171.6050136			

Table A24: Calculated Z-scores of Ara h 2.01 IgE inhibition experiments.

The calculated Z-scores of every peptide of Ara h 2.01_P and Ara h 2.01_Hyp are listed for the uninhibited serum pool (peanut patients 6, 7, 8, 10, 12, 15, 18, 21, 22 and 23) plus protein buffer without inhibitor (Pool -), for the serum pool preincubated with 13.5 μg rAra h 2.02 (Pool + rAra h 2.02), with 9.5 μg native peanut extract (Pool + n. extract) or preincubated with 9.5 μg reduced/alkylated peanut extract (Pool + r/a extract). Identified candidate diagnostic peptides are highlighted in light blue.

IgE inhibition experiment →	Pool -	Pool -	Pool + rAra h 2.02	Pool + rAra h 2.02	Pool + n. extract	Pool + n. extract	Pool + r/a extract	Pool + r/a extract
Ara h 2.01_P/Hyp	P	Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No. ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	4.122965	4.122965	0.557347	0.557347	2.998953	2.998953	2.882607	2.882607
2	95.36572	95.36572	1.192915	1.192915	3.017292	3.017292	14.3761	14.3761
3	8.055646	8.055646	0.284365	0.284365	2.082018	2.082018	2.142944	2.142944
4	2.653214	2.653214	-0.1449	-0.1449	1.673982	1.673982	1.769522	1.769522
5	1.633348	1.633348	0.092653	0.092653	2.19205	2.19205	2.010092	2.010092
6	1.448438	1.448438	0.169754	0.169754	1.536442	1.536442	1.690529	1.690529
7	1.059554	1.059554	0.125994	0.125994	2.320421	2.320421	3.453511	3.453511
8	1.091961	1.091961	-0.13448	-0.13448	1.568535	1.568535	1.428416	1.428416
9	66.54831	101.3172	0.044724	105.87	1.165083	5.923975	1.112443	5.826895
10	72.34725	101.7327	0.103072	104.4426	1.678567	9.92181	1.780294	10.47672
11	33.7353	101.8395	0.751143	101.5189	2.274574	7.400239	1.424825	9.622154
12	78.24531	99.80738	3.826877	57.0177	2.4763	1.811522	1.916736	1.733616
13	9.100294	92.41288	1.107478	18.06778	4.278077	1.513519	2.340427	0.843148
14	4.050526	4.967453	0.605275	0.253108	2.008663	1.027543	1.668985	0.642075
15	1.189182	1.189182	-0.21367	-0.21367	1.463087	1.463087	1.299154	1.299154
16	2.186173	2.186173	0.113491	0.113491	1.829861	1.829861	1.676167	1.676167
17	1.183463	1.183463	0.138497	0.138497	2.705534	2.705534	1.611536	1.611536
18	2.193798	2.193798	-0.01362	-0.01362	2.567994	2.567994	1.902374	1.902374
19	1.330248	1.330248	0.028054	0.028054	2.865997	2.865997	2.455326	2.455326
20	1.086242	1.086242	0.155167	0.155167	2.114111	2.114111	1.353013	1.353013
21	0.451447	0.451447	0.530257	0.530257	1.994909	1.994909	1.205799	1.205799
22	1.454157	1.454157	0.986616	0.986616	3.031046	3.031046	2.193212	2.193212
23	1.991731	1.991731	1.003287	1.003287	2.916429	2.916429	2.699486	2.699486
24	3.402387	3.402387	1.040796	1.040796	2.742211	2.742211	2.124991	2.124991
25	81.56226	81.56226	0.96161	0.96161	3.411573	3.411573	20.54475	20.54475
26	60.93046	60.93046	0.371886	0.371886	3.154832	3.154832	4.534281	4.534281
27	2.67609	2.67609	0.369802	0.369802	1.742752	1.742752	2.282977	2.282977
28	2.077514	2.077514	0.078066	0.078066	2.214974	2.214974	2.372742	2.372742
29	0.708797	0.708797	0.05306	0.05306	2.389191	2.389191	3.123177	3.123177
30	0.765985	0.765985	-0.01571	-0.01571	3.086062	3.086062	2.42301	2.42301
31	0.291319	0.291319	-0.07614	-0.07614	1.797768	1.797768	1.618717	1.618717
32	0.878457	0.878457	-0.08656	-0.08656	0.945019	0.945019	1.432006	1.432006
m(blanks)	6887.180488		9474.537374		19293.87429		14432.17866	
s(blanks)	524.5786309		479.8854558		218.1180411		278.5053352	

Table A25: Calculated Z-scores of Ara h 2.02 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each peanut-allergic patient (patients 1-3) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	1	1	2	2	3	3
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-15.6093	-15.6093	-15.837	-15.837	-16.689	-16.689
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	24.88522	24.88522	-5.86766	-5.86766	59.0829	59.0829
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-4.87386	-4.87386	-6.21678	-6.21678	-6.30131	-6.30131
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-7.00265	-7.00265	-7.65226	-7.65226	-7.58173	-7.58173
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-5.60232	-5.60232	-6.02318	-6.02318	-6.28988	-6.28988
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-5.24843	-5.24843	-5.48072	-5.48072	-6.5791	-6.5791
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-7.85529	-7.85529	-8.39668	-8.39668	-8.74548	-8.74548
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	-2.99305	-2.99305	-3.57739	-3.57739	-4.16075	-4.16075
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	-2.5275	53.079	-3.41774	-3.92737	-3.45695	105.7587
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	-1.7339	46.26586	-3.61779	-3.58699	-3.15993	108.3396
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	3.805916	82.91889	-3.85671	-3.21147	-2.90594	108.5642
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	-3.9494	26.18066	-6.06158	-3.42052	-6.23019	74.33564
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	-5.03707	1.970248	-5.9539	-4.27563	-5.52637	7.493399
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	-0.63999	44.22516	-3.5051	-4.35461	-1.96051	103.5128
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	16.62031	55.46646	-2.87767	-3.21141	-1.23041	74.49012
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	2.063132	69.57115	-5.03383	-5.31909	-3.06448	2.182899
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-6.5968	-2.46917	-8.52696	-5.31568	-7.2064	-4.87626
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-3.9879	-3.9879	-3.46818	-3.46818	-3.88176	-3.88176
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-6.72463	-6.72463	-6.81773	-6.81773	-6.73745	-6.73745
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-6.71527	-6.71527	-6.03765	-6.03765	-6.17534	-6.17534
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-3.90081	-3.90081	-3.53767	-3.53767	-3.70113	-3.70113
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-5.75533	-5.75533	-5.71415	-5.71415	-5.36392	-5.36392
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-3.08554	-3.08554	-2.63815	-2.63815	-3.23009	-3.23009
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-3.62827	-3.62827	-3.14614	-3.14614	-3.75113	-3.75113
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-2.80749	-2.80749	-2.71651	-2.71651	-3.13417	-3.13417
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-2.50519	-2.50519	-2.38013	-2.38013	-2.5867	-2.5867
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-1.95556	-1.95556	-2.7433	-2.7433	-3.26308	-3.26308
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-8.4171	-8.4171	-9.06065	-9.06065	-10.0842	-10.0842
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-6.36362	-6.36362	-7.90793	-7.90793	-8.20809	-8.20809
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-6.04957	-6.04957	-6.81515	-6.81515	-7.38515	-7.38515
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-10.0542	-10.0542	-10.4244	-10.4244	-10.7791	-10.7791
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-8.33437	-8.33437	-8.7882	-8.7882	-8.99561	-8.99561
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	-3.80065	-3.80065	-4.321	-4.321	-3.92286	-3.92286
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	-2.3958	-2.3958	-2.4209	-2.4209	-3.25623	-3.25623
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	-2.78834	-2.78834	-2.73773	-2.73773	-2.96914	-2.96914
	m (blanks)	9592.208333		10493.32271		8641.234136	
	s (blanks)	379.4304356		413.3282315		469.0740411	

Table A26: Calculated Z-scores of Ara h 2.02 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each peanut-allergic patient (patients 4-6) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	4	4	5	5	6	6
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-16.034	-16.034	-17.0706	-17.0706	-15.8523	-15.8523
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-7.44156	-7.44156	-5.58058	-5.58058	51.91572	51.91572
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-6.27436	-6.27436	-7.41301	-7.41301	-2.94865	-2.94865
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-6.96289	-6.96289	-7.92274	-7.92274	-7.97566	-7.97566
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-4.33781	-4.33781	-6.42141	-6.42141	-6.42816	-6.42816
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-4.02306	-4.02306	-6.18127	-6.18127	-6.15366	-6.15366
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-3.79996	-3.79996	-8.71047	-8.71047	-9.03132	-9.03132
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	-3.77511	-3.77511	-3.76773	-3.76773	-0.67626	-0.67626
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	-2.43561	-4.0963	-3.95888	-3.91921	56.1284	61.27734
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	-2.80977	-3.46961	-4.18443	-3.46427	55.12316	62.22379
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	-3.54256	-3.53794	-4.01635	-3.07345	57.86879	62.61336
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	-5.60294	-3.28695	-6.41272	-3.3469	54.88822	61.44064
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	-5.9148	-4.48906	-5.8809	-4.58721	1.197256	51.76891
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	-3.13846	-4.29	-3.35818	-4.20569	59.97855	60.91007
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	-2.22716	-3.3706	-3.07111	-3.37258	59.54316	60.71855
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	0.831244	-5.17911	-5.25413	-5.21392	-1.06828	44.31241
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-4.77099	-5.16686	-8.96274	-5.1901	-7.61904	-3.80097
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-3.16531	-3.16531	-3.94346	-3.94346	-3.75623	-3.75623
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-4.1665	-4.1665	-7.01191	-7.01191	-6.75581	-6.75581
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-5.94676	-5.94676	-6.29549	-6.29549	-6.8409	-6.8409
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-3.12277	-3.12277	-3.8991	-3.8991	-4.19802	-4.19802
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-1.87359	-1.87359	-6.02505	-6.02505	-6.34557	-6.34557
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-2.47114	-2.47114	-3.03512	-3.03512	-3.817	-3.817
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-3.10827	-3.10827	-3.31852	-3.31852	-4.3667	-4.3667
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-2.73123	-2.73123	-3.07695	-3.07695	-4.17304	-4.17304
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-1.51882	-1.51882	-2.84764	-2.84764	-4.03821	-4.03821
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-2.82996	-2.82996	-3.02953	-3.02953	-4.09812	-4.09812
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-8.7023	-8.7023	-10.1329	-10.1329	-9.64301	-9.64301
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-7.77342	-7.77342	-8.39344	-8.39344	-7.14176	-7.14176
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-6.62425	-6.62425	-7.29984	-7.29984	-6.34687	-6.34687
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-9.94065	-9.94065	-11.2671	-11.2671	-10.8884	-10.8884
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-5.14938	-5.14938	-9.31585	-9.31585	-9.07486	-9.07486
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	-2.96668	-2.96668	-4.58773	-4.58773	-3.42426	-3.42426
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	-2.51767	-2.51767	-2.82329	-2.82329	-3.42388	-3.42388
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	-2.79029	-2.79029	-2.74692	-2.74692	-3.6799	-3.6799
	m (blanks)	9287.655706		10237.31141		10105.10092	
	s (blanks)	467.2674622		444.6433874		799.5560648	

Table A27: Calculated Z-scores of Ara h 2.02 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each peanut-allergic patient (patients 7-9) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	7	7	8	8	9	9
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-14.4476	-14.4476	-15.9077	-15.9077	-17.2683	-17.2683
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	95.69471	95.69471	61.10624	61.10624	-8.33003	-8.33003
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	9.238021	9.238021	-3.8278	-3.8278	-7.47646	-7.47646
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-7.50257	-7.50257	-7.69436	-7.69436	-7.83942	-7.83942
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-6.38187	-6.38187	-6.09855	-6.09855	-6.13145	-6.13145
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-6.31751	-6.31751	-6.3266	-6.3266	-6.1345	-6.1345
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-7.90387	-7.90387	-9.27057	-9.27057	-8.72767	-8.72767
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	-3.94731	-3.94731	-3.67584	-3.67584	-3.88625	-3.88625
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	-3.26232	107.5682	-1.20208	72.88889	-3.56048	1.690126
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	11.40277	109.4753	3.825641	74.10853	-4.26198	1.192878
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	43.14967	109.815	2.911781	74.63243	-3.81688	3.656131
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	-5.7335	107.5882	-4.76436	73.87764	-6.58126	4.318492
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	-5.85832	57.57434	-5.08781	58.64393	-5.9236	-2.06095
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	-2.30512	107.226	9.883701	73.08679	-3.28466	3.988816
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	-1.90838	104.9979	51.1047	73.52093	-2.21743	3.255435
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-1.90809	47.56017	-1.74783	64.89771	-4.53255	0.801029
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-6.98664	-3.24033	-7.31896	-0.98894	-8.33918	-4.18569
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-2.82447	-2.82447	-4.04253	-4.04253	-3.92705	-3.92705
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-6.38536	-6.38536	-7.01856	-7.01856	-6.97447	-6.97447
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-6.44016	-6.44016	-6.72121	-6.72121	-6.48997	-6.48997
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-4.29073	-4.29073	-4.44403	-4.44403	-3.90429	-3.90429
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-6.24939	-6.24939	-6.53757	-6.53757	-5.92693	-5.92693
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-3.41191	-3.41191	-3.88966	-3.88966	-3.19863	-3.19863
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-3.92372	-3.92372	-4.32659	-4.32659	-3.79856	-3.79856
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-3.34883	-3.34883	-3.83497	-3.83497	-3.25885	-3.25885
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-2.84324	-2.84324	-3.80769	-3.80769	-2.73745	-2.73745
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-3.67502	-3.67502	-4.16047	-4.16047	-2.92809	-2.92809
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-8.2292	-8.2292	-9.46967	-9.46967	-9.78381	-9.78381
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-2.63336	-2.63336	-7.51587	-7.51587	-8.26953	-8.26953
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-6.89182	-6.89182	-7.12252	-7.12252	-7.24864	-7.24864
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-10.0466	-10.0466	-10.7588	-10.7588	-10.724	-10.724
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-8.5297	-8.5297	-8.68611	-8.68611	-8.56673	-8.56673
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	-4.54409	-4.54409	-3.64547	-3.64547	-4.45983	-4.45983
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	-3.36073	-3.36073	-3.31664	-3.31664	-2.93723	-2.93723
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	-3.05424	-3.05424	-3.39364	-3.39364	-3.1212	-3.1212
	m (blanks)	6735.093596		10444.14595		8851.646586	
	s (blanks)	479.5367787		668.9231327		450.4718604	

Table A28: Calculated Z-scores of Ara h 2.02 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each peanut-allergic patient (patients 10-12) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	10	10	11	11	12	12
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	34.90244	34.90244	-15.9818	-15.9818	-17.8098	-17.8098
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	77.86174	77.86174	-7.26357	-7.26357	13.83561	13.83561
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	53.44922	53.44922	-5.3545	-5.3545	-3.56168	-3.56168
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	7.806267	7.806267	-6.64606	-6.64606	-8.54491	-8.54491
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	3.38525	3.38525	-5.42017	-5.42017	-5.86026	-5.86026
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	26.37885	26.37885	-4.61825	-4.61825	-6.34004	-6.34004
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	2.068571	2.068571	-7.73704	-7.73704	-8.12188	-8.12188
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	7.138491	7.138491	-3.41502	-3.41502	-3.57547	-3.57547
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	50.31376	84.22832	-2.93219	-3.4073	6.592385	74.77807
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	72.32368	85.30851	-3.11666	-2.90797	33.6432	76.06382
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	66.51903	85.92153	-3.00255	-3.00092	42.40838	76.22799
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	50.78709	86.02486	-5.03779	-2.83771	-0.34555	75.0606
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	27.7416	84.33265	-5.21949	-3.75333	-5.07626	59.61445
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	83.10036	84.66779	-2.60149	-3.23415	36.83785	74.738
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	84.06202	85.20973	-2.23356	-2.14703	45.48413	74.67908
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	60.24778	82.81043	-2.91201	-4.98538	-2.40095	62.6302
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	29.58067	48.48469	-7.70878	-4.59509	-7.59642	-4.47731
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	3.14076	3.14076	-2.73329	-2.73329	-4.04453	-4.04453
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-2.61696	-2.61696	-5.30176	-5.30176	-7.15365	-7.15365
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-1.25619	-1.25619	-5.6368	-5.6368	-6.99015	-6.99015
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	1.889151	1.889151	-2.5663	-2.5663	-3.97514	-3.97514
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-1.04855	-1.04855	-4.3117	-4.3117	-6.11518	-6.11518
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	7.531052	7.531052	-1.80271	-1.80271	-3.2052	-3.2052
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	2.154222	2.154222	-2.02491	-2.02491	-3.8255	-3.8255
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	5.984522	5.984522	-1.65396	-1.65396	-3.44586	-3.44586
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	15.79713	15.79713	-1.43365	-1.43365	-3.0482	-3.0482
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	65.87531	65.87531	-1.72775	-1.72775	-2.8405	-2.8405
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	77.20752	77.20752	-9.6337	-9.6337	-9.67242	-9.67242
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	78.60053	78.60053	-7.29348	-7.29348	-8.90514	-8.90514
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	22.67847	22.67847	-6.33612	-6.33612	-8.29259	-8.29259
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	5.618181	5.618181	-10.2303	-10.2303	-11.5797	-11.5797
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	8.58615	8.58615	-6.90792	-6.90792	-9.54587	-9.54587
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	26.91765	26.91765	-3.5382	-3.5382	-4.03282	-4.03282
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	6.356191	6.356191	-2.08482	-2.08482	-3.06192	-3.06192
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	11.51741	11.51741	-1.55191	-1.55191	-3.01738	-3.01738
	m (blanks)	9362.16		8731.827506		10648.40678	
	s (blanks)	586.3116638		301.7226214		649.1258417	

Table A29: Calculated Z-scores of Ara h 2.02 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each peanut-allergic patient (patients 13-15) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	13	13	14	14	15	15
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-14.7109	-14.7109	-14.3288	-14.3288	-17.7549	-17.7549
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-6.53269	-6.53269	-5.55297	-5.55297	-1.04593	-1.04593
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-5.32674	-5.32674	-5.67415	-5.67415	-6.20294	-6.20294
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-6.27821	-6.27821	-5.90228	-5.90228	-6.9653	-6.9653
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-4.49461	-4.49461	-4.52588	-4.52588	-6.44673	-6.44673
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-4.40416	-4.40416	-4.17412	-4.17412	-5.35961	-5.35961
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-7.26123	-7.26123	-6.38768	-6.38768	-9.64886	-9.64886
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	-2.72192	-2.72192	-2.5178	-2.5178	-2.71822	-2.71822
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	-2.00622	-1.35399	-1.77138	-2.531	1.068816	79.90971
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	-2.3318	0.3073	-1.92091	-2.18041	29.87652	81.07799
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	-2.49577	0.066333	-2.37182	-1.83871	38.76177	81.38324
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	-4.8039	0.32162	-4.48874	-1.64348	22.02419	81.31837
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	-4.34112	-2.46701	-4.29739	-2.98603	2.572304	79.24477
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	-1.33105	0.982156	-1.36214	-2.48407	61.6226	80.16823
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	-0.60319	1.642752	-1.0109	-1.25573	37.38926	80.4774
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-2.42721	-3.5721	-2.66344	-3.22683	20.554	77.17393
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-5.97438	-4.0885	-7.10543	-3.92467	6.379057	9.471324
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-1.68813	-1.68813	-1.66895	-1.66895	-1.05645	-1.05645
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-4.68084	-4.68084	-5.00329	-5.00329	-6.15518	-6.15518
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-4.54011	-4.54011	-4.65663	-4.65663	-6.01381	-6.01381
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-1.35293	-1.35293	-1.74551	-1.74551	-2.57572	-2.57572
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-3.17075	-3.17075	-2.8896	-2.8896	-5.67057	-5.67057
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-0.02071	-0.02071	0.275816	0.275816	-3.3901	-3.3901
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-0.47059	-0.47059	-0.77225	-0.77225	-3.96937	-3.96937
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	0.138865	0.138865	-0.45385	-0.45385	-3.6236	-3.6236
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	0.667789	0.667789	0.84444	0.84444	-3.10565	-3.10565
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	0.539898	0.539898	0.61623	0.61623	-3.08725	-3.08725
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-7.87056	-7.87056	-7.8961	-7.8961	10.38215	10.38215
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-6.19471	-6.19471	-6.42128	-6.42128	70.46785	70.46785
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-5.96223	-5.96223	-5.55134	-5.55134	2.545822	2.545822
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-9.09529	-9.09529	-9.14142	-9.14142	-9.87605	-9.87605
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-6.4216	-6.4216	-6.27616	-6.27616	-8.034	-8.034
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	-2.23392	-2.23392	-1.61153	-1.61153	-2.77147	-2.77147
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	-0.64646	-0.64646	-0.17488	-0.17488	-3.13321	-3.13321
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	-0.38634	-0.38634	-0.16585	-0.16585	-2.6509	-2.6509
	m (blanks)	9020.593472		9055.216301		8750.54717	
	s (blanks)	199.1464024		198.9558915		626.7018591	

Table A30: Calculated Z-scores of Ara h 2.02 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each peanut-allergic patient (patients 16-18) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	16	16	17	17	18	18
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-16.5582	-16.5582	-16.3451	-16.3451	-16.0744	-16.0744
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-7.88047	-7.88047	73.6812	73.6812	93.44976	93.44976
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-6.79672	-6.79672	69.92526	69.92526	19.02107	19.02107
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-7.78089	-7.78089	-5.34555	-5.34555	-1.88096	-1.88096
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-5.58071	-5.58071	-6.04793	-6.04793	-1.47517	-1.47517
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-5.87647	-5.87647	-4.98714	-4.98714	3.853184	3.853184
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-7.18918	-7.18918	-9.06053	-9.06053	-6.0447	-6.0447
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	-3.88417	-3.88417	-4.33335	-4.33335	2.11779	2.11779
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	-3.25817	12.33794	-3.93747	62.30287	78.82741	107.5541
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	-3.51459	8.145488	-4.04732	77.20336	78.66647	108.9296
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	-3.45577	6.274935	-3.79449	76.275	84.739	109.3633
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	-6.02554	7.737454	-6.46408	76.65061	93.56194	109.1726
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	-5.21583	-1.69664	-5.62321	59.35653	20.20769	106.7326
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	-3.04125	14.80113	-0.07724	62.07124	103.6341	108.0219
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	-2.61734	21.69133	-2.19527	48.79285	106.82	108.4642
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-3.86616	2.28513	-4.43115	26.62258	88.44766	105.5339
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-7.82748	-4.6469	-8.35633	-3.27074	45.5348	66.87927
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-2.92936	-2.92936	-4.08974	-4.08974	5.658221	5.658221
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-6.17857	-6.17857	-7.13607	-7.13607	-5.22368	-5.22368
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-5.99052	-5.99052	-6.96013	-6.96013	-5.49551	-5.49551
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-2.88884	-2.88884	-3.81331	-3.81331	-1.56216	-1.56216
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-4.64394	-4.64394	-6.1348	-6.1348	-3.53141	-3.53141
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-2.09137	-2.09137	-3.44831	-3.44831	-2.26763	-2.26763
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-2.36326	-2.36326	-3.75596	-3.75596	-2.51895	-2.51895
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-1.99669	-1.99669	-3.23375	-3.23375	-2.46322	-2.46322
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-1.855	-1.855	-2.89908	-2.89908	-0.94734	-0.94734
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-1.90765	-1.90765	-2.81963	-2.81963	3.373468	3.373468
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-9.75742	-9.75742	-9.94956	-9.94956	48.1538	48.1538
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-7.98079	-7.98079	-7.40712	-7.40712	92.42276	92.42276
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-7.03544	-7.03544	-7.38833	-7.38833	2.326035	2.326035
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-10.9152	-10.9152	-11.1958	-11.1958	-6.0797	-6.0797
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-8.06822	-8.06822	-8.76832	-8.76832	-6.26779	-6.26779
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	-2.79967	-2.79967	-4.58202	-4.58202	17.70899	17.70899
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	-2.13639	-2.13639	-2.95943	-2.95943	-0.56794	-0.56794
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	-2.01333	-2.01333	-2.15571	-2.15571	4.860977	4.860977
	m (blanks)	9243.229314		11596.62551		10027.6129	
	s (blanks)	319.4069177		597.7429055		465.2042138	

Table A31: Calculated Z-scores of Ara h 2.02 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each peanut-allergic patient (patients 19-21) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	19	19	20	20	21	21
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-18.8479	-18.8479	-17.3958	-17.3958	-10.692	-10.692
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-9.49114	-9.49114	-6.4329	-6.4329	19.0295	19.0295
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-8.42145	-8.42145	-6.93501	-6.93501	13.57041	13.57041
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-8.77158	-8.77158	-8.27701	-8.27701	-5.72108	-5.72108
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-6.98684	-6.98684	-6.24875	-6.24875	-1.95257	-1.95257
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-6.83238	-6.83238	-6.1527	-6.1527	-0.91571	-0.91571
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-9.69779	-9.69779	-9.08661	-9.08661	-4.9797	-4.9797
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	-3.68507	-3.68507	-4.02065	-4.02065	17.86603	17.86603
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	-3.48435	-3.61603	-3.62969	64.50912	25.56568	25.72075
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	-3.60383	-1.79459	-3.76597	43.26565	25.20641	26.34223
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	-2.15981	-1.60427	-3.61184	23.75118	25.50286	26.50584
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	-6.25716	-3.11351	-5.98617	15.24659	22.91885	26.49105
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	-6.10546	-4.81832	-5.62803	-1.11197	22.27196	25.12514
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	-3.63024	-4.40949	-3.29726	48.99579	25.71304	25.2796
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	-3.27886	-3.74271	-2.65569	21.30513	25.9943	26.03679
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-5.60346	-5.98881	-4.82291	-1.36421	22.88784	23.93745
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-9.11512	-5.9428	-8.38959	-4.50856	9.59778	14.18211
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-3.8364	-3.8364	-3.68774	-3.68774	3.213946	3.213946
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-7.07964	-7.07964	-6.42866	-6.42866	-1.99622	-1.99622
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-6.79922	-6.79922	-6.31247	-6.31247	-2.80329	-2.80329
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-4.0151	-4.0151	-3.59415	-3.59415	-0.28184	-0.28184
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-6.06909	-6.06909	-5.35637	-5.35637	-3.97071	-3.97071
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-3.32256	-3.32256	-2.67321	-2.67321	-2.68056	-2.68056
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-3.91461	-3.91461	-3.04905	-3.04905	-1.86458	-1.86458
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-4.33977	-4.33977	-2.56758	-2.56758	-3.84413	-3.84413
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-4.13791	-4.13791	-2.10077	-2.10077	1.544414	1.544414
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-4.25733	-4.25733	-2.56136	-2.56136	-3.28397	-3.28397
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-11.4855	-11.4855	-9.94244	-9.94244	-2.78567	-2.78567
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-9.66175	-9.66175	-8.53565	-8.53565	-4.83594	-4.83594
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-8.42903	-8.42903	-7.12196	-7.12196	-5.8296	-5.8296
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-11.7138	-11.7138	-10.6796	-10.6796	-8.4868	-8.4868
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-9.51595	-9.51595	-8.86204	-8.86204	-6.96985	-6.96985
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	-4.76539	-4.76539	-3.82666	-3.82666	17.7496	17.7496
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	-2.91999	-2.91999	-2.45186	-2.45186	1.036338	1.036338
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	-3.39666	-3.39666	-2.33222	-2.33222	2.99955	2.99955
	m (blanks)	26168.36654		8583.473684		10659.28796	
	s (blanks)	9679.687758		342.5203501		1674.184356	

Table A32: Calculated Z-scores of Ara h 2.02 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each peanut-allergic patient (patients 22-23) and peanut tolerant patient 24 are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	22	22	23	23	24	24
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-16.6399	-16.6399	-15.6171	-15.6171	-15.6128	-15.6128
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-1.27362	-1.27362	62.1056	62.1056	-6.93763	-6.93763
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-6.23693	-6.23693	-3.79314	-3.79314	-6.10302	-6.10302
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-7.25223	-7.25223	-7.62967	-7.62967	-5.98791	-5.98791
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-5.94598	-5.94598	-6.10374	-6.10374	-5.26139	-5.26139
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-5.22242	-5.22242	-5.85019	-5.85019	-4.81836	-4.81836
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-9.05493	-9.05493	-9.1365	-9.1365	-7.30082	-7.30082
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	-2.67299	-2.67299	-1.72329	-1.72329	-3.81174	-3.81174
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	18.78786	83.51056	43.00367	77.58335	-3.65628	-3.57369
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	8.467562	84.87044	74.53287	78.84682	-3.21307	-2.64413
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	13.36838	85.17403	75.01098	79.14414	-3.37094	-2.67438
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	0.279667	84.53657	66.3178	76.41504	-5.1492	-2.928
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	-3.85541	78.73708	2.315558	54.56675	-5.17933	-4.35594
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	59.94622	83.5708	73.13518	76.60482	-3.37142	-4.01812
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	81.92574	83.26128	30.96051	75.19209	-2.48481	-2.88866
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	8.764985	78.6878	2.406967	60.40548	-4.51761	-4.82614
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-5.12402	1.574228	-7.26903	-2.91505	-8.00328	-4.56013
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-3.75026	-3.75026	-3.80419	-3.80419	-3.26279	-3.26279
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-6.92884	-6.92884	-6.88913	-6.88913	-5.90084	-5.90084
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-6.56524	-6.56524	-6.76973	-6.76973	-5.42116	-5.42116
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-3.98639	-3.98639	-4.3532	-4.3532	-3.27003	-3.27003
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-6.14108	-6.14108	-6.44635	-6.44635	-5.6705	-5.6705
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-3.57284	-3.57284	-3.9137	-3.9137	-2.30145	-2.30145
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-3.97929	-3.97929	-4.41712	-4.41712	-3.03544	-3.03544
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-3.83171	-3.83171	-4.11809	-4.11809	-2.44722	-2.44722
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-3.38208	-3.38208	-2.7914	-2.7914	-2.48563	-2.48563
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-3.52935	-3.52935	54.65392	54.65392	-2.59404	-2.59404
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-8.25758	-8.25758	0.726862	0.726862	-7.54432	-7.54432
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-7.0386	-7.0386	-6.25742	-6.25742	-6.91186	-6.91186
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-6.46838	-6.46838	-6.71567	-6.71567	-6.0481	-6.0481
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-10.2871	-10.2871	-10.5324	-10.5324	-9.99401	-9.99401
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-8.18412	-8.18412	-8.81047	-8.81047	-7.39489	-7.39489
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	-1.04865	-1.04865	-4.0538	-4.0538	-3.69785	-3.69785
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	-3.00073	-3.00073	-3.54872	-3.54872	-2.4918	-2.4918
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	-2.95311	-2.95311	-3.41797	-3.41797	-2.08355	-2.08355
	m (blanks)	9950.296073		9928.151057		11096.52677	
	s (blanks)	595.2847421		639.6703802		403.5663398	

Table A33: Calculated Z-scores of Ara h 2.02 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each peanut-tolerant patient (patients 25-27) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	25	25	26	26	27	27
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-14.403	-14.403	-20.1323	-20.1323	-15.8076	-15.8076
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-6.01015	-6.01015	-11.0327	-11.0327	-7.75596	-7.75596
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-4.21737	-4.21737	-9.42864	-9.42864	-6.5184	-6.5184
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-5.8365	-5.8365	-10.5685	-10.5685	-6.50266	-6.50266
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-2.75056	-2.75056	-8.66636	-8.66636	-5.51326	-5.51326
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-3.41084	-3.41084	-8.21585	-8.21585	-4.89704	-4.89704
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-4.66993	-4.66993	-10.4581	-10.4581	-5.48119	-5.48119
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	-1.79677	-1.79677	-4.92707	-4.92707	-3.66846	-3.66846
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	-1.18686	-1.14554	-4.79511	-5.12586	-3.57062	-3.39634
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	-1.27223	-1.56579	-5.57113	-4.56328	-3.53157	-2.98436
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	-2.128	-1.11102	-5.04653	-4.37128	-3.61886	-2.9536
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	-5.09737	-1.42652	-7.67512	-4.51794	-5.50608	-3.10037
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	-4.76824	-2.90976	-6.95129	-5.41135	-5.27544	-3.98097
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	-2.80302	-3.23118	-3.72821	-4.76003	-3.36815	-4.23602
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	-1.94835	-2.00659	-3.12656	-4.50188	-2.8941	-3.01485
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-0.99995	-2.55159	-4.747	-6.69918	-2.31304	-4.70111
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-5.90191	-2.28449	-8.72375	-6.42734	-5.73057	-4.61289
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-2.67444	-2.67444	-4.25869	-4.25869	-2.96116	-2.96116
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-4.9155	-4.9155	-6.65536	-6.65536	-4.50414	-4.50414
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-6.44484	-6.44484	-6.1806	-6.1806	-5.24058	-5.24058
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-3.0167	-3.0167	-3.12349	-3.12349	-3.09262	-3.09262
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-6.04653	-6.04653	-4.62019	-4.62019	-4.84881	-4.84881
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-3.10656	-3.10656	-1.16117	-1.16117	-2.69461	-2.69461
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-4.14433	-4.14433	-1.99643	-1.99643	-3.23534	-3.23534
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-4.03421	-4.03421	-0.39939	-0.39939	-2.35837	-2.35837
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-3.44764	-3.44764	-0.85233	-0.85233	-2.38317	-2.38317
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-4.00782	-4.00782	-0.56959	-0.56959	-2.73132	-2.73132
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-5.2364	-5.2364	-12.9257	-12.9257	-8.22009	-8.22009
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-4.88895	-4.88895	-11.0496	-11.0496	-6.83468	-6.83468
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-5.22516	-5.22516	-9.88632	-9.88632	-5.72654	-5.72654
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-8.234	-8.234	-13.1876	-13.1876	-9.69944	-9.69944
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-5.45557	-5.45557	-10.8999	-10.8999	-6.14743	-6.14743
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	-3.20642	-3.20642	-3.71805	-3.71805	-4.00955	-4.00955
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	-2.7347	-2.7347	-2.00099	-2.00099	-2.88625	-2.88625
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	-3.44037	-3.44037	-2.57086	-2.57086	-2.46464	-2.46464
	m (blanks)	12197.20253		14751.29909		11851.53209	
	s (blanks)	1714.718816		1597.646395		461.1748893	

Table A34: Calculated Z-scores of Ara h 2.02 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each peanut-tolerant patient (patients 28-30) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	28	28	29	29	30	30
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-16.1315	-16.1315	-16.1435	-16.1435	-15.9711	-15.9711
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-7.62222	-7.62222	-7.85828	-7.85828	-7.33023	-7.33023
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-6.51832	-6.51832	-6.36353	-6.36353	-6.01509	-6.01509
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-7.1998	-7.1998	-6.85042	-6.85042	-7.36758	-7.36758
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-5.88483	-5.88483	-5.49648	-5.49648	-5.17322	-5.17322
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-5.37518	-5.37518	-5.67097	-5.67097	-5.48496	-5.48496
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-7.81996	-7.81996	-8.11885	-8.11885	-7.48527	-7.48527
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	-3.69535	-3.69535	-3.59731	-3.59731	-3.20791	-3.20791
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	-3.49115	-3.62824	-3.27909	-3.27572	-3.03397	-2.58144
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	-3.70136	-3.24205	-3.77391	-3.16094	-2.61581	-2.45374
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	-3.65632	-2.95437	-3.64009	-2.67947	-3.03246	-2.44833
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	-5.30146	-3.35017	-6.09627	-2.80763	-5.13155	-2.57004
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	-5.52756	-4.46523	-5.8605	-4.17182	-5.35814	-3.59143
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	-3.23503	-4.38033	-3.24756	-4.06172	-3.12249	-3.59327
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	-2.53177	-3.53275	-3.06847	-3.05561	-2.38001	-2.52177
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-4.27148	-5.02536	-4.76046	-4.34831	-4.51062	-3.90443
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-8.21094	-4.92154	-8.42008	-4.79801	-8.56386	-3.83761
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-3.66215	-3.66215	-3.48713	-3.48713	-3.19686	-3.19686
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-6.32261	-6.32261	-6.81214	-6.81214	-6.37968	-6.37968
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-5.95125	-5.95125	-6.45309	-6.45309	-6.22553	-6.22553
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-3.14778	-3.14778	-3.77435	-3.77435	-3.46751	-3.46751
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-4.51958	-4.51958	-5.66493	-5.66493	-5.56529	-5.56529
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-2.33378	-2.33378	-2.62856	-2.62856	-2.58738	-2.58738
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-3.03036	-3.03036	-3.31512	-3.31512	-3.03907	-3.03907
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-2.53202	-2.53202	-2.97149	-2.97149	-3.11186	-3.11186
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-2.26669	-2.26669	-2.66838	-2.66838	-2.34768	-2.34768
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-2.94247	-2.94247	-3.2777	-3.2777	-2.67929	-2.67929
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-8.78904	-8.78904	-8.71254	-8.71254	-8.29625	-8.29625
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-7.47421	-7.47421	-7.13505	-7.13505	-6.33661	-6.33661
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-6.09591	-6.09591	-6.60001	-6.60001	-5.74918	-5.74918
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-10.1136	-10.1136	-9.76832	-9.76832	-9.37562	-9.37562
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-8.27336	-8.27336	-8.33809	-8.33809	-6.6277	-6.6277
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	-4.03221	-4.03221	-4.21291	-4.21291	-3.91574	-3.91574
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	-2.59168	-2.59168	-2.56094	-2.56094	-2.35896	-2.35896
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	-2.51655	-2.51655	-2.64113	-2.64113	-2.72016	-2.72016
	m (blanks)	12315.06472		14543.81696		10913.91392	
	s (blanks)	500.0454477		384.1710475		327.9266085	

Table A35: Calculated Z-scores of Ara h 2.02 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each peanut-tolerant patient (patients 31-33) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	31	31	32	32	33	33
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-16.7887	-16.7887	-17.6274	-17.6274	-17.2259	-17.2259
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-8.26537	-8.26537	-9.33768	-9.33768	-8.87163	-8.87163
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-7.21115	-7.21115	-7.48401	-7.48401	-7.74605	-7.74605
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-7.50519	-7.50519	-8.15567	-8.15567	-8.1327	-8.1327
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-5.76727	-5.76727	-6.25402	-6.25402	-6.31786	-6.31786
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-5.36563	-5.36563	-6.01389	-6.01389	-6.26277	-6.26277
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-7.98976	-7.98976	-8.45314	-8.45314	-8.70007	-8.70007
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	-3.74177	-3.74177	-3.60259	-3.60259	-3.32361	-3.32361
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	-3.11096	-3.82048	-3.53286	-3.63712	-3.46966	-3.23009
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	-3.87964	-3.42876	-3.69769	-3.29237	-4.08521	-2.98918
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	-3.23456	-3.11786	-3.48462	-2.95103	-3.52998	-2.85084
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	-5.73338	-3.24004	-5.86751	-2.93437	-5.89253	-2.81504
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	-5.38817	-4.34514	-5.22774	-4.24214	-5.47836	-3.8711
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	-2.8756	-4.13089	-3.38196	-4.05179	-3.07563	-3.79069
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	-2.53524	-3.4115	-2.46068	-2.98029	-2.71924	-3.01664
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-4.28348	-5.14944	-4.7494	-5.12299	-4.74462	-4.93864
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-7.46789	-5.10172	-8.23087	-5.31731	-8.20714	-5.23644
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-3.30554	-3.30554	-3.56242	-3.56242	-3.44765	-3.44765
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-6.22196	-6.22196	-6.68035	-6.68035	-6.32937	-6.32937
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-6.01436	-6.01436	-6.0449	-6.0449	-6.11147	-6.11147
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-2.99621	-2.99621	-3.52031	-3.52031	-3.64677	-3.64677
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-4.84845	-4.84845	-5.27068	-5.27068	-5.33036	-5.33036
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-2.33978	-2.33978	-2.60911	-2.60911	-2.71434	-2.71434
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-2.46843	-2.46843	-3.07917	-3.07917	-2.84428	-2.84428
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-2.30882	-2.30882	-2.66893	-2.66893	-2.48194	-2.48194
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-1.56976	-1.56976	-2.20574	-2.20574	-2.23401	-2.23401
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-1.58082	-1.58082	-2.62377	-2.62377	-2.60475	-2.60475
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-9.70918	-9.70918	-10.1905	-10.1905	-10.0671	-10.0671
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-8.15775	-8.15775	-8.71408	-8.71408	-8.68301	-8.68301
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-7.50076	-7.50076	-7.40233	-7.40233	-7.62749	-7.62749
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-10.8529	-10.8529	-10.8681	-10.8681	-10.9929	-10.9929
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-8.66204	-8.66204	-8.77289	-8.77289	-8.81125	-8.81125
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	-4.02328	-4.02328	-4.092	-4.092	-3.81373	-3.81373
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	-2.43007	-2.43007	-2.73686	-2.73686	-2.48301	-2.48301
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	-2.58709	-2.58709	-2.32541	-2.32541	-2.34124	-2.34124
	m (blanks)	12100.85777		11909.87841		10981.51739	
	s (blanks)	429.7547642		444.6552176		408.3888554	

Table A36: Calculated Z-scores of Ara h 2.02 peptides after control subtraction.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each peanut-tolerant patient (patients 34-35) are listed. Identified candidate diagnostic peptides are highlighted in light blue. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Patient No. →	34	34	35	35
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	-17.8392	-17.8392	-17.6687	-17.6687
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	-9.23519	-9.23519	-9.06935	-9.06935
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	-7.68299	-7.68299	-7.94408	-7.94408
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	-8.78861	-8.78861	-8.12819	-8.12819
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	-6.77961	-6.77961	-6.44503	-6.44503
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	-7.07231	-7.07231	-6.37625	-6.37625
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	-9.6506	-9.6506	-9.02647	-9.02647
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	-4.04325	-4.04325	-3.78481	-3.78481
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	-3.59317	-3.50408	-3.75328	-3.59785
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	-4.24893	-2.97865	-4.3359	-3.12068
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	-4.17696	-2.72633	-4.08341	-2.81681
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	-6.19456	-2.85261	-6.57513	-2.87537
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	-5.88062	-3.6198	-5.7963	-3.95325
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	-3.54907	-4.02637	-3.48704	-3.82419
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	-2.28729	-2.80698	-2.96976	-3.21163
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	-3.89956	-4.71507	-5.56081	-4.73287
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	-7.72748	-5.63145	-8.82928	-4.91599
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	-3.83128	-3.83128	-4.13538	-4.13538
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	-6.68164	-6.68164	-6.94447	-6.94447
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	-6.77929	-6.77929	-6.5081	-6.5081
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-4.40655	-4.40655	-4.12249	-4.12249
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	-6.03547	-6.03547	-5.79592	-5.79592
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	-2.6388	-2.6388	-3.05105	-3.05105
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	-3.72641	-3.72641	-3.42906	-3.42906
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	-3.69882	-3.69882	-2.84092	-2.84092
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	-3.35388	-3.35388	-2.8154	-2.8154
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	-3.81816	-3.81816	-3.23611	-3.23611
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	-10.5205	-10.5205	-10.2296	-10.2296
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	-8.51213	-8.51213	-8.55121	-8.55121
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	-7.67201	-7.67201	-7.68731	-7.68731
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	-11.328	-11.328	-10.7786	-10.7786
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	-9.15491	-9.15491	-9.04834	-9.04834
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	-3.78171	-3.78171	-3.91315	-3.91315
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	-2.61758	-2.61758	-2.66655	-2.66655
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	-3.25986	-3.25986	-2.5693	-2.5693
	m (blanks)	12530.63095		10368.17722	
	s (blanks)	506.3960279		455.1615403	

Table A37: Calculated Z-scores of Ara h 2.02 peptides of controls.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each control are listed. Numbers written behind control sera represent the respective developed X-ray film. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Control No. →	N_2	N_2	N_3	N_3	N_4	N_4
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	2.618215	2.618215	2.289753	2.289753	3.022174	3.022174
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	2.46384	2.46384	1.745793	1.745793	2.937296	2.937296
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	2.178675	2.178675	1.569007	1.569007	2.746965	2.746965
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	2.208692	2.208692	1.617963	1.617963	2.394596	2.394596
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	2.324473	2.324473	1.66148	1.66148	2.152824	2.152824
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	2.315897	2.315897	0.899936	0.899936	1.671852	1.671852
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	2.789743	2.789743	1.481973	1.481973	1.952205	1.952205
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	0.553448	0.553448	0.296141	0.296141	0.6096	0.6096
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	0.482693	-0.17126	0.608918	-0.06287	0.666185	0.50929
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	0.950106	0.056017	0.546363	-0.21246	0.751062	0.529866
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	0.956539	0.028144	0.551802	-0.23966	0.542727	0.419269
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	1.168804	0.051729	0.598039	-0.04111	0.902812	0.607028
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	0.289724	0.049585	0.328779	0.054079	0.421841	0.465565
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	0.279004	-0.23129	0.508286	-0.11183	0.48357	0.311243
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	0.435523	-0.28489	0.875458	0.116635	0.3704	0.329247
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	2.255862	0.257563	2.390385	0.200948	1.455801	1.468661
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	2.045741	0.017424	2.512776	-0.07647	1.257754	1.059707
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	1.170949	1.170949	1.174636	1.174636	0.671329	0.671329
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	1.597624	1.597624	1.131119	1.131119	0.535011	0.535011
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	1.040159	1.040159	0.826502	0.826502	0.411553	0.411553
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	1.014429	1.014429	0.98969	0.98969	0.645608	0.645608
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	0.920089	0.920089	0.85098	0.85098	0.691905	0.691905
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	0.514854	0.514854	1.579886	1.579886	0.625032	0.625032
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	0.825749	0.825749	0.823782	0.823782	0.694477	0.694477
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	0.881495	0.881495	1.234472	1.234472	0.828223	0.828223
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	1.32318	1.32318	2.053131	2.053131	0.398692	0.398692
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	0.892216	0.892216	1.688678	1.688678	0.645608	0.645608
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	1.434673	1.434673	2.009614	2.009614	2.422888	2.422888
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	1.546166	1.546166	1.949778	1.949778	2.574639	2.574639
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	1.514004	1.514004	2.080329	2.080329	2.129676	2.129676
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	1.218119	1.218119	1.626122	1.626122	2.157968	2.157968
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	2.009291	2.009291	1.751233	1.751233	1.988213	1.988213
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	-0.00831	-0.00831	1.182795	1.182795	0.542727	0.542727
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	-0.04047	-0.04047	0.793864	0.793864	0.535011	0.535011
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	0.049585	0.049585	0.668754	0.668754	0.437273	0.437273
	m (blanks)	9170.873662		8143.116379		11455.98985	
	s (blanks)	466.3965312		367.6744314		388.7963548	

Table A38: Calculated Z-scores of Ara h 2.02 peptides of controls.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each control are listed. Numbers written behind control sera represent the respective developed X-ray film. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Control No. →	DLab71S1_1	DLab71S1_1	DLab71S1_2	DLab71S1_2	DLab71S1_3	DLab71S1_3
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	2.490168	2.490168	2.887099	2.887099	2.550297	2.550297
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	1.48453	1.48453	2.551398	2.551398	2.118537	2.118537
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	1.005075	1.005075	2.418741	2.418741	2.128406	2.128406
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	0.633293	0.633293	2.080332	2.080332	0.976222	0.976222
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	1.04977	1.04977	1.768996	1.768996	1.193336	1.193336
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	1.02539	1.02539	1.590316	1.590316	0.976222	0.976222
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	1.482499	1.482499	1.936847	1.936847	1.000894	1.000894
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	0.023815	0.023815	0.688794	0.688794	0.240995	0.240995
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	0.564219	-0.3886	1.373734	0.518236	0.724567	0.194118
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	0.643451	-0.64255	0.864767	0.361215	0.601207	0.206454
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	0.048195	-0.39469	0.501993	0.279996	0.285405	0.502519
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	0.306207	-0.5369	0.515529	0.515529	0.238528	0.221258
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	0.127427	-0.23623	0.718574	0.558845	0.134905	0.680157
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	0.296049	-0.21998	0.407238	0.225851	0.28047	-0.10441
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	0.29808	0.332617	0.501993	0.331435	0.440839	0.566666
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	1.48453	-0.09808	1.9937	1.495562	1.588088	0.512388
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	1.370761	0.076637	1.766289	0.518236	1.889087	0.396429
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	0.446387	0.446387	1.113836	1.113836	1.15386	1.15386
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	0.657672	0.657672	0.918913	0.918913	0.618477	0.618477
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	0.99898	0.99898	0.680673	0.680673	0.376691	0.376691
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	1.027422	1.027422	0.518236	0.518236	0.603674	0.603674
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	1.204171	1.204171	1.113836	1.113836	1.158795	1.158795
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	1.393109	1.393109	0.761891	0.761891	0.640682	0.640682
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	0.812073	0.812073	0.242095	0.242095	0.455642	0.455642
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	1.722227	1.722227	1.062398	1.062398	0.645616	0.645616
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	1.447962	1.447962	1.084056	1.084056	1.225409	1.225409
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	1.571889	1.571889	1.154445	1.154445	0.949082	0.949082
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	1.244802	1.244802	3.017048	3.017048	2.261635	2.261635
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	1.261055	1.261055	2.602836	2.602836	2.345519	2.345519
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	0.8974	0.8974	2.17238	2.17238	1.935964	1.935964
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	0.517492	0.517492	1.741923	1.741923	1.437589	1.437589
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	1.324034	1.324034	2.296914	2.296914	2.505888	2.505888
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	0.560156	0.560156	0.612991	0.612991	0.576535	0.576535
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	0.470766	0.470766	0.109438	0.109438	0.260733	0.260733
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	0.239164	0.239164	0.144633	0.144633	0.32488	0.32488
	m (blanks)	11243.27745		11721.57619		9079.320513	
	s (blanks)	492.2247585		369.3755052		405.3172388	

Table A39: Calculated Z-scores of Ara h 2.02 peptides of controls.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each control are listed. Numbers written behind control sera represent the respective developed X-ray film. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Control No. →	DLab71S1_4	DLab71S1_4	DLab72S1_1	DLab72S1_1	DLab72S1_2	DLab72S1_2
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	1.554046	1.554046	1.91215	1.91215	2.307903	2.307903
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	1.661996	1.661996	0.798671	0.798671	1.742894	1.742894
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	2.303697	2.303697	1.347073	1.347073	1.591359	1.591359
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	1.627512	1.627512	0.704182	0.704182	1.420341	1.420341
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	1.137241	1.137241	0.66157	0.66157	0.82719	0.82719
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	0.828385	0.828385	0.291028	0.291028	1.032844	1.032844
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	2.723501	2.723501	1.085841	1.085841	1.097788	1.097788
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	0.444564	0.444564	-0.1036	-0.1036	0.305476	0.305476
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	1.670992	0.165694	0.207656	-0.30925	0.396397	0.205896
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	0.735429	0.056246	0.077966	-0.42968	0.563086	0.244862
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	0.598992	0.008268	0.002005	-0.5112	0.591228	0.229709
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	1.723467	-0.08619	0.437392	-0.11472	0.515461	0.002406
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	0.029258	0.038254	0.103904	-0.26108	0.350937	0.231874
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	0.171692	0.273644	0.263237	-0.18697	0.251357	0.255686
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	0.416078	-0.11018	0.294733	0.378105	0.17126	0.457011
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	1.042785	0.834382	1.185887	-0.04431	0.92677	0.699467
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	0.141706	0.669459	0.596725	-0.14065	0.935429	0.47
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	0.41158	0.41158	0.291028	0.291028	0.6237	0.6237
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	0.254153	0.254153	0.498531	0.498531	0.686479	0.686479
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	3.705542	3.705542	0.433687	0.433687	0.335783	0.335783
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	-0.00523	-0.00523	0.828314	0.828314	0.513296	0.513296
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	0.551015	0.551015	0.909833	0.909833	0.539273	0.539273
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	0.318623	0.318623	1.002469	1.002469	0.396397	0.396397
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	0.048749	0.048749	0.568934	0.568934	0.580404	0.580404
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	0.416078	0.416078	1.076577	1.076577	0.467835	0.467835
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	0.387591	0.387591	1.178476	1.178476	0.359596	0.359596
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	0.210673	0.210673	1.302608	1.302608	0.664831	0.664831
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	3.834482	3.834482	1.636096	1.636096	1.745059	1.745059
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	1.576536	1.576536	1.967731	1.967731	1.548063	1.548063
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	2.140273	2.140273	0.991352	0.991352	1.257982	1.257982
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	0.912346	0.912346	0.454066	0.454066	1.766706	1.766706
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	1.52556	1.52556	1.247026	1.247026	1.322925	1.322925
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	0.02476	0.02476	0.961709	0.961709	0.22105	0.22105
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	-0.16715	-0.16715	0.66157	0.66157	0.16693	0.16693
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	-0.12067	-0.12067	0.289175	0.289175	0.374749	0.374749
	m (blanks)	11903.48548		11209.91781		11229.88839	
	s (blanks)	666.977806		539.7497324		461.9395702	

Table A40: Calculated Z-scores of Ara h 2.02 peptides of controls and derived maximum Z-score.

The calculated Z-scores of every peptide of Ara h 2.02_P and Ara h 2.02_Hyp for each control and the maximum Z-score (Max.) of the controls are listed. Numbers written behind control sera represent the respective developed X-ray film. Prolines undergoing post-translational hydroxylation are written in bold and blue.

	Control No. →	DLab72S1_3	DLab72S1_3	DLab72S1_4	DLab72S1_4	Max.	Max.
	Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	R-Q-Q-W-E-L-Q-G-D-R-R-C-Q-S-Q	2.898064	2.898064	18.05717	18.05717	18.05717	18.05717
2	E-L-Q-G-D-R-R-C-Q-S-Q-L-E-R-A	2.144974	2.144974	9.391927	9.391927	9.391927	9.391927
3	D-R-R-C-Q-S-Q-L-E-R-A-N-L-R-P	2.429927	2.429927	7.970057	7.970057	7.970057	7.970057
4	Q-S-Q-L-E-R-A-N-L-R-P-C-E-Q-H	1.663268	1.663268	8.459553	8.459553	8.459553	8.459553
5	E-R-A-N-L-R-P-C-E-Q-H-L-M-Q-K	1.699452	1.699452	6.600633	6.600633	6.600633	6.600633
6	L-R-P-C-E-Q-H-L-M-Q-K-I-Q-R-D	0.926009	0.926009	6.45495	6.45495	6.45495	6.45495
7	E-Q-H-L-M-Q-K-I-Q-R-D-E-D-S-Y	1.84419	1.84419	9.467682	9.467682	9.467682	9.467682
8	M-Q-K-I-Q-R-D-E-D-S-Y-G-R-D-P	0.328965	0.328965	3.949196	3.949196	3.949196	3.949196
9	Q-R-D-E-D-S-Y-G-R-D-P-Y-S-**P**-S	1.014209	-0.13239	3.820994	3.716102	3.820994	3.716102
10	D-S-Y-G-R-D-P-Y-S-**P**-S-Q-D-P-Y	0.812933	-0.08263	4.287181	3.249915	4.287181	3.249915
11	R-D-P-Y-S-**P**-S-Q-D-P-Y-S-**P**-S-Q	0.249811	-0.02836	3.98416	3.104232	3.98416	3.104232
12	S-**P**-S-Q-D-P-Y-S-**P**-S-Q-D-P-D-R	0.860425	-0.04871	6.513223	3.110059	6.513223	3.110059
13	D-P-Y-S-**P**-S-Q-D-P-D-R-R-D-P-Y	0.326703	0.021397	6.035382	4.357109	6.035382	4.357109
14	**P**-S-Q-D-P-D-R-R-D-P-Y-S-**P**-S-P	0.116381	-0.06454	3.622865	4.252217	3.622865	4.252217
15	P-D-R-R-D-P-Y-S-**P**-S-P-Y-D-R-R	0.55738	0.213627	3.26157	3.360635	3.26157	3.360635
16	D-P-Y-S-**P**-S-P-Y-D-R-R-G-A-G-S	1.789914	0.575472	5.703224	5.231209	5.703224	5.231209
17	**P**-S-P-Y-D-R-R-G-A-G-S-S-Q-H-Q	1.165731	0.168396	9.094733	5.225382	9.094733	5.225382
18	D-R-R-G-A-G-S-S-Q-H-Q-E-R-C-C	0.663672	0.663672	3.989987	3.989987	3.989987	3.989987
19	A-G-S-S-Q-H-Q-E-R-C-C-N-E-L-N	0.797102	0.797102	7.148403	7.148403	7.148403	7.148403
20	Q-H-Q-E-R-C-C-N-E-L-N-E-F-E-N	1.127285	1.127285	6.857036	6.857036	6.857036	6.857036
21	R-C-C-N-E-L-N-E-F-E-N-N-Q-R-C	1.631607	1.631607	4.07157	4.07157	4.07157	4.07157
22	E-L-N-E-F-E-N-N-Q-R-C-M-C-E-A	0.559641	0.559641	6.10531	6.10531	6.10531	6.10531
23	F-E-N-N-Q-R-C-M-C-E-A-L-Q-Q-I	0.299565	0.299565	3.26157	3.26157	3.26157	3.26157
24	Q-R-C-M-C-E-A-L-Q-Q-I-M-E-N-Q	0.091504	0.091504	3.646174	3.646174	3.646174	3.646174
25	C-E-A-L-Q-Q-I-M-E-N-Q-S-D-R-L	0.607133	0.607133	3.5238	3.5238	3.5238	3.5238
26	Q-Q-I-M-E-N-Q-S-D-R-L-Q-G-R-Q	0.844594	0.844594	3.366462	3.366462	3.366462	3.366462
27	E-N-Q-S-D-R-L-Q-G-R-Q-Q-E-Q-Q	0.659148	0.659148	3.710275	3.710275	3.710275	3.710275
28	D-R-L-Q-G-R-Q-Q-E-Q-Q-F-K-R-E	2.016067	2.016067	10.71473	10.71473	10.71473	10.71473
29	G-R-Q-Q-E-Q-Q-F-K-R-E-L-R-N-L	2.158543	2.158543	9.053941	9.053941	9.053941	9.053941
30	E-Q-Q-F-K-R-E-L-R-N-L-P-Q-Q-C	1.457469	1.457469	7.917611	7.917611	7.917611	7.917611
31	K-R-E-L-R-N-L-P-Q-Q-C-G-L-R-A	2.429927	2.429927	11.43732	11.43732	11.43732	11.43732
32	R-N-L-P-Q-Q-C-G-L-R-A-P-Q-R-C	2.090697	2.090697	9.351136	9.351136	9.351136	9.351136
33	Q-Q-C-G-L-R-A-P-Q-R-C-D-L-E-V	0.419426	0.419426	4.59603	4.59603	4.59603	4.59603
34	L-R-A-P-Q-R-C-D-L-E-V-E-S-G-G	0.344796	0.344796	2.993513	2.993513	2.993513	2.993513
35	Q-R-C-D-L-E-V-E-S-G-G-R-D-R-Y	0.148043	0.148043	2.964376	2.964376	2.964376	2.964376
	m (blanks)	8431.538776		10277.29825			
	s (blanks)	442.1784235		171.6050136			

Table A41: Calculated Z-scores of Ara h 2.02 IgE inhibition experiments.

The calculated Z-scores of every peptide of Ara h 2.02_P/Hyp are listed for the uninhibited peanut serum pool plus protein buffer without inhibitor (Pool -), for the peanut serum pool preincubated with rAra h 2.02 (Pool + rAra h 2.02), with native peanut extract (Pool + n. extract) or with reduced/alkylated peanut extract (Pool + r/a extract). Candidate diagnostic peptides are highlighted in light blue.

IgE inhibition experiment →	Pool -	Pool -	Pool + rAra h 2.02	Pool + rAra h 2.02	Pool + n. extract	Pool + n. extract	Pool + r/a extract	Pool + r/a extract
Ara h 2.02_P/Hyp	P	Hyp	P	Hyp	P	Hyp	P	Hyp
Peptide No. ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	4.12296534	4.12296534	0.55734681	0.55734681	2.99895282	2.99895282	2.88260668	2.88260668
2	95.3657213	95.3657213	1.19291514	1.19291514	3.01729151	3.01729151	14.3761028	14.3761028
3	8.05564555	8.05564555	0.284365	0.284365	2.08201812	2.08201812	2.14294401	2.14294401
4	2.65321428	2.65321428	-0.14490411	-0.14490411	1.67398218	1.67398218	1.76952208	1.76952208
5	1.6333481	1.6333481	0.09265258	0.09265258	2.19205029	2.19205029	2.01009198	2.01009198
6	1.44843779	1.44843779	0.16975431	0.16975431	1.53644198	1.53644198	1.69052898	1.69052898
7	1.05955424	1.05955424	0.12599387	0.12599387	2.32042114	2.32042114	3.45351136	3.45351136
8	1.43128116	1.43128116	0.29686798	0.29686798	0.90834171	0.90834171	0.72465879	0.72465879
9	3.13550613	100.747183	0.11765855	100.672904	1.03671257	3.7279159	0.93291333	3.37092767
10	6.95762141	101.412479	0.25935903	101.81276	1.59145806	6.20822426	1.80542803	4.83589063
11	8.78956793	101.62789	0.18017347	102.019059	1.59604273	6.74004638	1.42841551	10.1679249
12	3.18316343	100.737652	0.29478415	76.32126	1.56395002	1.33930102	1.45354968	1.1160337
13	2.2223923	89.4714667	0.42189782	13.1249292	1.41265579	1.25677689	1.84133399	0.30455912
14	14.7257609	101.784206	0.39897568	100.816689	2.14161888	8.97736705	1.29197288	8.34749301
15	40.4025598	99.8416947	0.86575374	80.6452085	2.35709853	2.99436815	1.48945563	1.91673649
16	9.10029351	92.4128751	1.10747809	18.0677754	4.27807672	1.51351861	2.34042676	0.84314844
17	4.05052625	4.96745265	0.60527491	0.25310754	2.00866335	1.02754322	1.66898541	0.6420751
18	1.18918209	1.18918209	-0.21367052	-0.21367052	1.4630872	1.4630872	1.29915407	1.29915407
19	2.18617276	2.18617276	0.11349089	0.11349089	1.82986108	1.82986108	1.6761666	1.6761666
20	1.18346321	1.18346321	0.13849685	0.13849685	2.70553372	2.70553372	1.61153588	1.61153588
21	2.19379793	2.19379793	-0.01362278	-0.01362278	2.56799351	2.56799351	1.90237411	1.90237411
22	1.33024769	1.33024769	0.02805383	0.02805383	2.86599729	2.86599729	2.45532582	2.45532582
23	1.08624233	1.08624233	0.1551675	0.1551675	2.11411084	2.11411084	1.353013	1.353013
24	0.45144712	0.45144712	0.53025701	0.53025701	1.99490933	1.99490933	1.20579859	1.20579859
25	1.45415666	1.45415666	0.98661591	0.98661591	3.03104553	3.03104553	2.19321235	2.19321235
26	1.99173098	1.99173098	1.00328656	1.00328656	2.9164287	2.9164287	2.69948631	2.69948631
27	3.402387	3.402387	1.04079551	1.04079551	2.7422111	2.7422111	2.12499103	2.12499103
28	81.5622616	81.5622616	0.96160994	0.96160994	3.41157343	3.41157343	20.5447459	20.5447459
29	60.9304642	60.9304642	0.37188588	0.37188588	3.15483172	3.15483172	4.53428061	4.53428061
30	2.67608978	2.67608978	0.36980205	0.36980205	1.74275228	1.74275228	2.28297723	2.28297723
31	2.07751412	2.07751412	0.07806577	0.07806577	2.21497365	2.21497365	2.37274212	2.37274212
32	0.70879653	0.70879653	0.0530598	0.0530598	2.38919125	2.38919125	3.12317658	3.12317658
33	1.22349534	1.22349534	0.1551675	0.1551675	2.66427165	2.66427165	1.4320061	1.4320061
34	0.48576037	0.48576037	0.06973044	0.06973044	1.85278445	1.85278445	0.82160487	0.82160487
35	1.06146053	1.06146053	0.45732294	0.45732294	1.74733696	1.74733696	1.36737539	1.36737539
m(blanks)	6887.180488		9474.537374		19293.87429		14432.17866	
s(blanks)	524.5786309		479.8854558		218.1180411		278.5053352	

7.1.5.3 Calculated Z-scores of Pis s 1 peptides

Table A42: Calculated Z-scores of Pis s 1 peptides after control subtraction.

The calculated Z-scores of every peptide of Pis s 1 for each pea-allergic patient (patients 1-7) are listed. Identified candidate diagnostic peptides are highlighted in light blue.

	Patient No. →	1	2	3	4	5	6	7
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	S-R-S-D-Q-E-N-P-F-I-F-K-S-N-R	-10.5309	-9.46465	-10.6778	-3.96957	-10.5275	-4.72091	-8.02724
2	Q-E-N-P-F-I-F-K-S-N-R-F-Q-T-L	-12.2751	-11.603	-13.3058	-5.03865	-11.0193	-4.0631	-3.89503
3	F-I-F-K-S-N-R-F-Q-T-L-Y-E-N-E	2.090892	-0.06186	0.411962	12.82627	1.535048	7.696264	2.78552
4	S-N-R-F-Q-T-L-Y-E-N-E-N-G-H-I	2.612442	1.410022	3.63417	14.99363	1.626418	4.782956	2.551252
5	Q-T-L-Y-E-N-E-N-G-H-I-R-L-L-Q	3.218103	2.369175	0.768995	8.559985	0.889885	3.289293	1.558609
6	E-N-E-N-G-H-I-R-L-L-Q-K-F-D-K	-4.45952	-4.53468	-5.94043	-5.48877	-7.48853	-2.22716	-3.45339
7	G-H-I-R-L-L-Q-K-F-D-K-R-S-K-I	-30.1759	-24.3011	-26.6026	-23.0893	-23.5681	-2.10184	-4.64472
8	L-L-Q-K-F-D-K-R-S-K-I-F-E-N-L	-1.26056	-2.89415	-1.82134	8.728744	-2.76111	1.457342	-0.29759
9	F-D-K-R-S-K-I-F-E-N-L-Q-N-Y-R	-13.7991	-14.1687	-12.8785	-6.1962	-12.1531	-8.53486	-7.34012
10	S-K-I-F-E-N-L-Q-N-Y-R-L-L-E-Y	-0.74305	-1.95426	-0.02966	2.408014	-2.28009	2.064805	-0.99076
11	E-N-L-Q-N-Y-R-L-L-E-Y-K-S-K-P	-6.08846	-5.61855	-3.95807	-3.09112	-5.59648	-0.35238	0.230783
12	N-Y-R-L-L-E-Y-K-S-K-P-H-T-L-F	-8.80944	-7.74146	-5.17451	-2.67718	-7.25844	-3.78246	-2.11138
13	L-E-Y-K-S-K-P-H-T-L-F-L-P-Q-Y	-8.34012	-8.12264	-5.17606	3.634752	-9.21854	3.465189	-1.01692
14	S-K-P-H-T-L-F-L-P-Q-Y-T-D-A-D	2.511129	-1.94265	1.425025	21.60567	-2.22342	8.543576	2.066228
15	T-L-F-L-P-Q-Y-T-D-A-D-F-I-L-V	1.6304	-2.14891	-0.29177	24.7479	-3.15707	11.12796	3.16967
16	P-Q-Y-T-D-A-D-F-I-L-V-V-L-S-G	-1.9238	-5.39107	-1.8642	13.27394	-4.84575	8.481657	0.790868
17	D-A-D-F-I-L-V-V-L-S-G-K-A-T-L	-15.9675	-15.5858	-12.3471	0.736501	-15.6619	13.68749	7.331134
18	I-L-V-V-L-S-G-K-A-T-L-T-V-L-K	-9.53085	-10.1468	-8.39849	4.16522	-12.6191	6.84126	4.686985
19	L-S-G-K-A-T-L-T-V-L-K-S-N-D-R	-1.82265	-4.66876	-1.16883	12.07631	-6.54259	2.503649	4.507498
20	A-T-L-T-V-L-K-S-N-D-R-N-S-F-N	0.538323	-3.72059	-1.29529	11.76476	-3.61255	13.19017	6.946854
21	V-L-K-S-N-D-R-N-S-F-N-L-E-R-G	-1.62549	-2.47007	-2.28448	12.7868	-2.40583	5.450079	3.410066
22	N-D-R-N-S-F-N-L-E-R-G-D-A-I-K	-3.06508	-5.0999	-0.56215	10.92419	-4.46687	3.565495	3.049704
23	S-F-N-L-E-R-G-D-A-I-K-L-P-A-G	-0.40525	-3.97911	-2.37106	13.7626	-2.59786	3.114325	0.451875
24	E-R-G-D-A-I-K-L-P-A-G-T-I-A-Y	0.232908	-2.77959	-1.46504	10.90085	-1.27212	5.389901	19.44002
25	A-I-K-L-P-A-G-T-I-A-Y-L-A-N-R	-6.38797	-6.99169	-8.2296	3.126432	-7.30122	-5.33415	-7.43907
26	P-A-G-T-I-A-Y-L-A-N-R-D-D-N-E	-0.01261	-0.28158	-3.72475	7.981769	-1.1882	4.027554	-2.38786
27	I-A-Y-L-A-N-R-D-D-N-E-D-L-R-V	-2.9128	-3.08892	-5.73733	9.482944	-3.14504	9.754615	0.774336
28	A-N-R-D-D-N-E-D-L-R-V-L-D-L-A	5.777238	0.722055	2.264753	9.964865	3.022396	9.532204	2.756042
29	D-N-E-D-L-R-V-L-D-L-A-I-P-V-N	5.999491	0.412868	0.259976	13.56267	3.979282	8.395579	3.44504
30	L-R-V-L-D-L-A-I-P-V-N-K-P-G-Q	6.693737	1.136713	1.915241	11.56879	8.145987	5.719216	6.273872
31	D-L-A-I-P-V-N-K-P-G-Q-L-Q-S-F	4.193609	-1.39301	-0.34959	8.864606	-3.81689	3.205944	-0.83328
32	P-V-N-K-P-G-Q-L-Q-S-F-L-L-S-G	4.984036	-0.34499	2.356422	7.068273	-0.52217	2.509449	0.013138
33	P-G-Q-L-Q-S-F-L-L-S-G-T-Q-N-Q	-1.2832	-0.21224	-4.34667	8.960293	-0.95953	3.518867	-0.63075
34	Q-S-F-L-L-S-G-T-Q-N-Q-P-S-L-L	3.833144	0.260894	0.799559	13.25493	2.054154	5.86136	3.72952
35	L-S-G-T-Q-N-Q-P-S-L-L-S-G-F-S	-1.37214	-3.24364	-2.85876	9.368696	-2.76493	1.334022	-1.7238
36	Q-N-Q-P-S-L-L-S-G-F-S-K-N-I-L	-1.63033	1.204264	-0.06499	5.776599	-2.77938	0.495834	2.673118
37	S-L-L-S-G-F-S-K-N-I-L-E-A-A-F	1.793282	-1.45992	3.869664	14.72835	-1.02973	1.752846	3.100017

38	G-F-S-K-N-I-L-E-A-A-F-N-T-N-Y	1.383962	-2.71259	0.82768	18.08994	-2.83587	3.577405	2.871174
39	N-I-L-E-A-A-F-N-T-N-Y-E-E-I-E	0.192607	-2.9097	-0.26251	15.39546	-2.65176	3.129273	-0.97128
40	A-A-F-N-T-N-Y-E-E-I-E-K-V-L-L	-0.05999	-2.45204	0.365505	21.33392	-2.94183	5.979964	1.290693
41	T-N-Y-E-E-I-E-K-V-L-L-E-Q-Q-E	-1.73178	-1.43261	-0.72888	11.03196	-3.76814	2.067562	-0.19162
42	E-I-E-K-V-L-L-E-Q-Q-E-Q-E-P-Q	-0.55314	-2.12327	1.571556	8.507774	-2.79875	2.613184	1.279057
43	V-L-L-E-Q-Q-E-Q-E-P-Q-H-R-R-S	0.731218	-1.95218	3.41436	6.017752	-2.13356	2.689308	3.15173
44	Q-Q-E-Q-E-P-Q-H-R-R-S-L-K-D-R	0.52806	-1.85856	4.557498	10.78453	-2.81785	2.838024	5.190264
45	E-P-Q-H-R-R-S-L-K-D-R-R-Q-E-I	-17.095	-12.7173	-11.5967	-7.40311	-8.76291	9.615661	8.774303
46	R-R-S-L-K-D-R-R-Q-E-I-N-E-E-N	0.117823	-2.17983	-1.11531	9.596477	-1.9904	5.08288	6.417096
47	K-D-R-R-Q-E-I-N-E-E-N-V-I-V-K	-1.33484	-4.92204	-3.19539	7.217198	-3.46168	4.426172	-0.06936
48	Q-E-I-N-E-E-N-V-I-V-K-V-S-R-E	2.134784	-1.57343	-0.43696	18.88174	-0.32714	12.44053	3.241052
49	E-E-N-V-I-V-K-V-S-R-E-Q-I-E-E	1.566656	-0.00766	-2.82063	7.302274	-0.49477	1.891113	-3.07897
50	I-V-K-V-S-R-E-Q-I-E-E-L-S-K-N	2.076582	1.78776	-2.79039	9.790269	0.726109	2.70424	-1.67731
51	S-R-E-Q-I-E-E-L-S-K-N-A-K-S-S	2.48216	-0.23984	-0.91134	5.671486	2.944714	2.328423	-0.93638
52	I-E-E-L-S-K-N-A-K-S-S-S-K-K-S	-13.3178	-10.8975	-9.99684	-8.41396	-9.15579	-1.8459	-1.78169
53	S-K-N-A-K-S-S-S-K-K-S-V-S-S-E	2.741248	-1.93334	0.320026	12.49301	5.107093	3.377901	3.276868
54	K-S-S-S-K-K-S-V-S-S-E-S-G-P-F	6.064404	-1.36414	0.637707	17.33392	1.800313	5.567717	2.010493
55	K-K-S-V-S-S-E-S-G-P-F-N-L-R-S	8.633018	0.22755	2.252867	22.5075	3.135521	3.994672	4.040989
56	S-S-E-S-G-P-F-N-L-R-S-R-N-P-I	2.288639	-2.29369	-0.65917	15.19035	-1.29167	0.728345	0.519631
57	G-P-F-N-L-R-S-R-N-P-I-Y-S-N-K	-37.9551	-33.3032	-34.6763	-12.4847	-26.0553	0.193242	-11.278
58	L-R-S-R-N-P-I-Y-S-N-K-F-G-K-F	-40.6655	-36.2857	-39.2972	-16.3835	-19.9754	14.85147	-12.9861
59	N-P-I-Y-S-N-K-F-G-K-F-F-E-I-T	3.059435	-1.07734	-3.17006	25.6919	-2.50029	3.304393	-0.16243
60	S-N-K-F-G-K-F-F-E-I-T-P-E-K-N	4.625283	0.383156	-0.38702	25.10105	-0.62787	1.023545	0.732993
61	G-K-F-F-E-I-T-P-E-K-N-Q-Q-L-Q	2.296988	-1.05604	1.48857	28.40486	-0.24523	3.74048	-0.02319
62	E-I-T-P-E-K-N-Q-Q-L-Q-D-L-D-I	4.227438	-1.33692	3.698734	30.95001	-1.3195	3.865156	1.072578
63	E-K-N-Q-Q-L-Q-D-L-D-I-F-V-N-S	7.560559	-1.18087	2.020102	34.20865	-1.14768	5.885953	2.27638
64	Q-L-Q-D-L-D-I-F-V-N-S-V-D-I-K	5.914372	-0.53136	6.961027	33.82082	-1.73688	8.502636	2.31189
65	L-D-I-F-V-N-S-V-D-I-K-E-G-S-L	3.320574	-0.45831	2.979496	25.30886	-0.73421	5.281205	1.377918
66	V-N-S-V-D-I-K-E-G-S-L-L-L-P-N	3.021156	-2.65594	1.303705	22.4685	-0.53752	7.863785	4.808514
67	D-I-K-E-G-S-L-L-L-P-N-Y-N-S-R	-1.98571	-3.97336	-0.56915	16.75311	-2.88654	1.041249	2.252869
68	G-S-L-L-L-P-N-Y-N-S-R-A-I-V-I	-1.34385	-4.02649	-1.68399	11.27882	-4.11514	4.175076	3.510956
69	L-P-N-Y-N-S-R-A-I-V-I-V-T-V-T	-4.01863	-3.78902	-2.151	13.15501	-2.28686	12.8682	7.42568
70	N-S-R-A-I-V-I-V-T-V-T-E-G-K-G	-3.33638	-5.78534	-2.32991	22.20884	-4.53755	13.45803	7.663475
71	I-V-I-V-T-V-T-E-G-K-G-D-F-E-L	6.889609	-0.55084	5.806017	33.60079	0.205413	7.160616	5.219416
72	T-V-T-E-G-K-G-D-F-E-L-V-G-Q-R	5.071344	-0.93334	1.903718	32.05923	-1.99385	8.846595	5.354521
73	G-K-G-D-F-E-L-V-G-Q-R-N-E-N-Q	5.296378	-1.83315	-2.68609	4.781424	-1.54813	-0.12328	-4.45089
74	F-E-L-V-G-Q-R-N-E-N-Q-G-K-E-N	6.422961	4.390234	-0.39038	12.36706	1.63479	4.479886	0.045552
75	G-Q-R-N-E-N-Q-G-K-E-N-D-K-E-E	2.609272	0.753812	1.70486	3.816325	-1.4771	0.415805	-2.32193
76	E-N-Q-G-K-E-N-D-K-E-E-E-Q-E-E	0.970757	-0.51274	7.668478	2.268469	-1.41979	-0.40678	-1.10332
77	K-E-N-D-K-E-E-E-Q-E-E-E-T-S-K	4.931304	0.966154	7.760659	7.389933	-0.49591	1.12645	0.501257
78	K-E-E-E-Q-E-E-E-T-S-K-Q-V-Q-L	8.456177	-0.30665	3.032057	11.15886	1.473152	5.23006	0.211462
79	Q-E-E-E-T-S-K-Q-V-Q-L-Y-R-A-K	8.10252	0.932493	0.491612	13.86043	-0.14372	1.731035	0.436978
80	T-S-K-Q-V-Q-L-Y-R-A-K-L-S-P-G	0.813897	-3.45831	-3.43447	21.9388	-3.57547	5.890986	3.192777
81	V-Q-L-Y-R-A-K-L-S-P-G-D-V-F-V	-0.29545	-3.90783	-3.40332	28.22442	0.46693	3.585538	0.360856
82	R-A-K-L-S-P-G-D-V-F-V-I-P-A-G	8.508339	0.855691	2.415826	34.96222	4.900988	6.389808	-0.45357

83	S-P-G-D-V-F-V-I-P-A-G-H-P-V-A	10.84649	1.232026	7.501944	37.34794	2.456817	3.783556	0.272683
84	V-F-V-I-P-A-G-H-P-V-A-I-N-A-S	9.406568	0.531597	3.753001	35.6474	5.576492	9.005023	1.194817
85	P-A-G-H-P-V-A-I-N-A-S-S-D-L-N	5.596367	-1.51501	0.276765	29.25137	0.045064	7.567014	-0.061
86	P-V-A-I-N-A-S-S-D-L-N-L-I-G-F	6.021637	-1.53364	-3.42973	32.75131	0.764875	7.08186	-1.00746
87	N-A-S-S-D-L-N-L-I-G-F-G-I-N-A	6.788298	-4.68605	-0.48836	33.05649	0.422313	2.871794	-3.28233
88	D-L-N-L-I-G-F-G-I-N-A-E-N-N-E	8.90907	-2.06978	1.42784	35.78641	-1.31983	5.843576	0.291208
89	I-G-F-G-I-N-A-E-N-N-E-R-N-F-L	5.253261	-1.18743	0.173815	32.75868	-0.45967	10.28601	0.558966
90	I-N-A-E-N-N-E-R-N-F-L-A-G-E-E	-2.83805	-13.9276	-10.7819	21.99911	21.79074	-2.9373	-10.3445
91	N-N-E-R-N-F-L-A-G-E-E-D-N-V-I	7.1123	-3.47123	-0.87748	33.9903	6.032384	2.825362	2.211427
92	N-F-L-A-G-E-E-D-N-V-I-S-Q-V-E	0.856132	-2.47024	1.788573	18.12826	-0.66382	4.155449	0.089739
93	G-E-E-D-N-V-I-S-Q-V-E-R-P-V-K	-0.41212	-2.16006	-1.31281	15.93288	-0.90224	5.68074	2.892422
94	N-V-I-S-Q-V-E-R-P-V-K-E-L-A-F	4.120173	-0.98495	3.757626	32.63297	1.570174	8.318921	3.999988
95	Q-V-E-R-P-V-K-E-L-A-F-P-G-S-S	5.588146	13.77671	11.52922	32.186	7.391694	3.001639	2.205856
96	P-V-K-E-L-A-F-P-G-S-S-H-E-V-D	8.202078	14.98353	13.98078	34.63	34.81028	8.781856	60.48037
97	L-A-F-P-G-S-S-H-E-V-D-R-L-L-K	8.159771	0.120814	12.59132	6.97535	3.450639	0.860178	-0.3118
98	G-S-S-H-E-V-D-R-L-L-K-N-Q-K-Q	5.883925	-0.92573	0.011273	1.876871	-1.71492	-1.09575	-2.85998
99	E-V-D-R-L-L-K-N-Q-K-Q-S-Y-F-A	1.126948	-1.19532	-3.44025	25.66664	-2.46879	-0.57718	-6.55732
100	L-L-K-N-Q-K-Q-S-Y-F-A-N-A-Q-P	7.300018	0.949288	13.7509	32.67821	-0.43074	-0.77202	6.237889
101	Q-K-Q-S-Y-F-A-N-A-Q-P-L-Q-R-E	7.730445	0.627144	13.301	32.55821	12.73965	3.627507	6.965832
	m (blanks)	20535.63	14861.14	14342.39	11846.79	9434.028	9808.343	10057.57
	s (blanks)	3476.642	2354.966	2687.228	1345.152	1206.837	518.3151	657.7803

Table A43: Calculated Z-scores of Pis s 1 peptides after control subtraction.

The calculated Z-scores of every peptide of Pis s 1 for each pea-allergic patient (patients 8-14) are listed. Identified candidate diagnostic peptides are highlighted in light blue.

	Patient No. →	8	9	10	11	12	13	14
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	S-R-S-D-Q-E-N-P-F-I-F-K-S-N-R	-10.2048	-10.2714	-9.94922	-8.09417	-11.5047	-8.92543	-9.85826
2	Q-E-N-P-F-I-F-K-S-N-R-F-Q-T-L	-6.63055	-11.1714	-10.64	-5.0821	-15.4895	-10.3766	-9.32076
3	F-I-F-K-S-N-R-F-Q-T-L-Y-E-N-E	8.983865	-0.8895	2.478525	7.866443	-2.10504	2.140051	-0.40102
4	S-N-R-F-Q-T-L-Y-E-N-E-N-G-H-I	4.108445	0.027464	2.580308	4.79595	0.135541	1.168597	0.798883
5	Q-T-L-Y-E-N-E-N-G-H-I-R-L-L-Q	3.888052	-0.70903	1.187946	2.109534	-0.13531	0.463244	-0.10134
6	E-N-E-N-G-H-I-R-L-L-Q-K-F-D-K	-4.05861	-7.89764	-6.51231	-6.49825	-7.73976	-5.38584	-5.11145
7	G-H-I-R-L-L-Q-K-F-D-K-R-S-K-I	-25.4841	-22.152	-29.9044	-25.844	-33.6198	-27.5886	-26.9745
8	L-L-Q-K-F-D-K-R-S-K-I-F-E-N-L	8.398056	-4.8384	-1.2943	-3.20085	-6.14205	-2.5384	-5.04725
9	F-D-K-R-S-K-I-F-E-N-L-Q-N-Y-R	-9.22516	-13.2744	-14.6752	-12.951	-16.7983	-13.1335	-14.4459
10	S-K-I-F-E-N-L-Q-N-Y-R-L-L-E-Y	1.460095	-2.04811	-1.79529	-1.11854	-2.07226	-1.46783	-2.3407
11	E-N-L-Q-N-Y-R-L-L-E-Y-K-S-K-P	-3.89541	-4.81998	-5.08657	-6.57906	-6.38108	-4.20365	-4.47754
12	N-Y-R-L-L-E-Y-K-S-K-P-H-T-L-F	0.031794	-7.6524	-6.16626	-7.07201	-10.0257	-5.99142	-6.66043
13	L-E-Y-K-S-K-P-H-T-L-F-L-P-Q-Y	1.974939	-7.26183	-7.2484	-6.99326	-10.2088	-5.58832	-6.97463
14	S-K-P-H-T-L-F-L-P-Q-Y-T-D-A-D	17.17633	-2.21045	0.593432	3.014678	-2.4796	0.528871	-3.27262
15	T-L-F-L-P-Q-Y-T-D-A-D-F-I-L-V	17.45499	-2.72388	-0.68258	2.881373	-4.63346	-0.51519	-4.95449
16	P-Q-Y-T-D-A-D-F-I-L-V-V-L-S-G	8.61695	-3.97002	-4.1381	-0.29107	-6.13263	-2.19202	-5.74932
17	D-A-D-F-I-L-V-V-L-S-G-K-A-T-L	3.528927	-17.0695	-16.641	-8.81724	-19.8498	-15.3455	-16.4854

18	I-L-V-V-L-S-G-K-A-T-L-T-V-L-K	8.386515	-8.65533	-10.0874	-0.54583	-13.6171	-6.6154	-9.89171
19	L-S-G-K-A-T-L-T-V-L-K-S-N-D-R	13.02	-4.70983	-2.95544	2.800711	1.404287	0.269636	-3.14796
20	A-T-L-T-V-L-K-S-N-D-R-N-S-F-N	14.24501	-2.15239	-0.55992	3.305823	2.513176	0.87497	0.602229
21	V-L-K-S-N-D-R-N-S-F-N-L-E-R-G	7.90013	-2.75799	-1.94478	4.626593	3.501281	3.559365	-0.01419
22	N-D-R-N-S-F-N-L-E-R-G-D-A-I-K	19.59179	-5.19495	-1.41857	3.450057	-5.40825	-0.63176	-3.4624
23	S-F-N-L-E-R-G-D-A-I-K-L-P-A-G	9.633813	-4.26439	0.21289	0.836571	-4.55191	-2.05431	-4.16214
24	E-R-G-D-A-I-K-L-P-A-G-T-I-A-Y	7.062155	-2.60887	1.431596	-0.33469	-3.0962	-2.0213	-3.63413
25	A-I-K-L-P-A-G-T-I-A-Y-L-A-N-R	-2.4057	-7.14246	-5.72097	-4.04999	-8.83641	-7.07549	-6.7774
26	P-A-G-T-I-A-Y-L-A-N-R-D-D-N-E	3.772197	-2.25676	0.615739	4.712762	-4.27999	-1.70977	-2.50015
27	I-A-Y-L-A-N-R-D-D-N-E-D-L-R-V	0.621597	-3.31672	-1.32753	5.434854	-7.39504	-3.76042	-5.09878
28	A-N-R-D-D-N-E-D-L-R-V-L-D-L-A	6.021402	0.095972	2.921814	6.650664	-0.97558	0.290552	-0.21075
29	D-N-E-D-L-R-V-L-D-L-A-I-P-V-N	15.78444	-0.54929	3.851405	10.92319	-1.54138	1.269412	-0.7978
30	L-R-V-L-D-L-A-I-P-V-N-K-P-G-Q	19.31143	0.354649	5.490765	11.33449	0.026469	2.788748	-0.39833
31	D-L-A-I-P-V-N-K-P-G-Q-L-Q-S-F	-2.02424	-2.63852	0.27718	-1.22192	-2.7221	1.509109	-3.62289
32	P-V-N-K-P-G-Q-L-Q-S-F-L-L-S-G	12.88187	-1.32515	1.260762	4.093779	-1.26598	2.194086	-1.97283
33	P-G-Q-L-Q-S-F-L-L-S-G-T-Q-N-Q	8.842039	-4.89652	-0.33243	0.240076	-4.9309	-2.88297	-4.71059
34	Q-S-F-L-L-S-G-T-Q-N-Q-P-S-L-L	18.40619	0.262881	2.34371	5.120569	-2.00076	1.648636	-2.11005
35	L-S-G-T-Q-N-Q-P-S-L-L-S-G-F-S	11.14791	-3.78764	-2.7025	0.922758	-4.57934	-3.16056	-4.70801
36	Q-N-Q-P-S-L-L-S-G-F-S-K-N-I-L	7.946933	-2.35556	1.473322	-2.18899	-2.74653	-0.74923	-2.13444
37	S-L-L-S-G-F-S-K-N-I-L-E-A-A-F	15.78799	-1.14448	0.606908	3.826505	-1.68147	0.568944	-1.91552
38	G-F-S-K-N-I-L-E-A-A-F-N-T-N-Y	15.29004	-2.46356	0.505527	2.702483	-3.31008	0.577059	-3.3423
39	N-I-L-E-A-A-F-N-T-N-Y-E-E-I-E	8.252952	-2.53346	-1.37604	0.89492	-2.48333	-0.95087	-3.75072
40	A-A-F-N-T-N-Y-E-E-I-E-K-V-L-L	16.64576	-2.11701	-1.58657	4.250589	-3.15764	-0.50757	-4.44183
41	T-N-Y-E-E-I-E-K-V-L-L-E-Q-Q-E	10.6009	-1.74394	-2.48714	2.08073	-3.3836	-0.6547	-3.50978
42	E-I-E-K-V-L-L-E-Q-Q-E-Q-E-P-Q	13.17028	-2.17601	-0.27544	5.060719	-2.55652	0.368662	-1.82639
43	V-L-L-E-Q-Q-E-Q-E-P-Q-H-R-R-S	17.31133	-1.90373	2.067706	7.633024	-1.90579	0.881792	0.163845
44	Q-Q-E-Q-E-P-Q-H-R-R-S-L-K-D-R	12.5985	-2.17513	0.079773	7.106346	-2.3981	2.794986	1.672636
45	E-P-Q-H-R-R-S-L-K-D-R-R-Q-E-I	2.73805	-9.62477	-12.6639	-9.06075	-18.1558	-9.32395	-9.76644
46	R-R-S-L-K-D-R-R-Q-E-I-N-E-E-N	15.2471	-2.90992	0.521375	4.098121	-2.58212	0.511729	-1.8243
47	K-D-R-R-Q-E-I-N-E-E-N-V-I-V-K	12.93871	-4.7385	-0.59554	1.533438	-4.94487	-3.35532	-5.2501
48	Q-E-I-N-E-E-N-V-I-V-K-V-S-R-E	20.09891	-1.64398	4.269022	4.56753	-2.73756	0.02588	-2.89223
49	E-E-N-V-I-V-K-V-S-R-E-Q-I-E-E	1.694651	-0.72585	1.06947	3.68999	-3.0576	-1.71992	-2.80153
50	I-V-K-V-S-R-E-Q-I-E-E-L-S-K-N	7.013111	-1.49431	2.63883	7.854344	-3.17143	-1.87566	-2.66663
51	S-R-E-Q-I-E-E-L-S-K-N-A-K-S-S	9.638157	-1.57953	2.617368	8.954074	-3.74712	-0.85498	-2.21744
52	I-E-E-L-S-K-N-A-K-S-S-S-K-K-S	-10.8657	-10.7079	-14.967	-4.81964	-18.7811	-12.3053	-13.1299
53	S-K-N-A-K-S-S-S-K-K-S-V-S-S-E	9.058007	-3.4792	-0.98639	11.97582	-5.59819	-1.77073	-3.54114
54	K-S-S-S-K-K-S-V-S-S-E-S-G-P-F	6.699502	-1.5265	1.944281	9.563492	-2.42419	-0.42862	-2.71156
55	K-K-S-V-S-S-E-S-G-P-F-N-L-R-S	12.10288	-0.14942	3.881052	7.71819	-1.10517	1.551206	-1.79729
56	S-S-E-S-G-P-F-N-L-R-S-R-N-P-I	17.98396	-2.91603	-0.79933	0.424173	-4.70548	-0.49276	-4.82885
57	G-P-F-N-L-R-S-R-N-P-I-Y-S-N-K	-9.28238	-11.5012	-35.305	-28.1298	-42.665	-35.7518	-36.6245
58	L-R-S-R-N-P-I-Y-S-N-K-F-G-K-F	-16.65	-20.08	-40.3601	-29.8161	-48.1048	-40.1486	-40.8516
59	N-P-I-Y-S-N-K-F-G-K-F-F-E-I-T	27.1435	-2.96516	-3.57233	4.332919	-4.49684	-2.07623	-4.69066
60	S-N-K-F-G-K-F-F-E-I-T-P-E-K-N	24.63675	-0.77676	-1.03938	4.498331	-1.86813	0.169367	-1.92672
61	G-K-F-F-E-I-T-P-E-K-N-Q-Q-L-Q	20.15338	-0.92707	0.591618	2.891999	-1.87374	-0.3005	-2.21619
62	E-I-T-P-E-K-N-Q-Q-L-Q-D-L-D-I	28.44564	-1.62879	2.781417	6.869579	-2.53588	1.749469	-2.79209

63	E-K-N-Q-Q-L-Q-D-L-D-I-F-V-N-S	29.28988	-0.98768	1.333496	8.843022	-2.33452	1.787476	-2.82543
64	Q-L-Q-D-L-D-I-F-V-N-S-V-D-I-K	32.0517	-0.49256	2.7884	15.84811	-2.33056	1.877137	-2.79892
65	L-D-I-F-V-N-S-V-D-I-K-E-G-S-L	24.69747	0.02791	0.811937	7.150099	-1.34354	2.092048	-1.56679
66	V-N-S-V-D-I-K-E-G-S-L-L-L-P-N	29.8012	-1.26868	2.467722	12.34733	-2.87538	2.375586	-2.21317
67	D-I-K-E-G-S-L-L-L-P-N-Y-N-S-R	31.18535	-3.08199	0.448639	6.670182	-4.17719	0.493556	-1.61979
68	G-S-L-L-L-P-N-Y-N-S-R-A-I-V-I	25.27933	-2.71278	-3.16121	7.848274	-6.43439	-1.48581	-3.59476
69	L-P-N-Y-N-S-R-A-I-V-I-V-T-V-T	21.12295	-2.43589	-0.89521	6.134128	-7.21908	-0.06419	-3.74802
70	N-S-R-A-I-V-I-V-T-V-T-E-G-K-G	20.72911	-6.12752	-0.45205	3.547835	-9.30162	-3.94506	-7.1857
71	I-V-I-V-T-V-T-E-G-K-G-D-F-E-L	37.83927	-0.53358	6.512703	16.23704	-2.28169	2.185225	-1.95453
72	T-V-T-E-G-K-G-D-F-E-L-V-G-Q-R	29.70321	-0.90949	6.682954	16.53343	-3.02598	0.744416	-2.81495
73	G-K-G-D-F-E-L-V-G-Q-R-N-E-N-Q	10.6583	-1.89841	2.553099	4.978139	-4.99448	-2.99661	-4.55669
74	F-E-L-V-G-Q-R-N-E-N-Q-G-K-E-N	12.95894	-0.55264	5.292882	8.747864	-1.84911	-0.30131	-1.06078
75	G-Q-R-N-E-N-Q-G-K-E-N-D-K-E-E	8.396359	-1.33827	3.525614	5.505535	-3.25194	-2.01695	-2.24295
76	E-N-Q-G-K-E-N-D-K-E-E-E-Q-E-E	7.312862	-1.04291	0.495388	4.720078	-2.11764	-1.35775	-2.37024
77	K-E-N-D-K-E-E-E-Q-E-E-E-T-S-K	20.805	1.357316	2.984326	14.53277	-1.71907	0.227568	-1.72269
78	K-E-E-E-Q-E-E-E-T-S-K-Q-V-Q-L	18.32081	0.605867	1.542492	7.730916	-1.54122	0.586685	-1.50817
79	Q-E-E-E-T-S-K-Q-V-Q-L-Y-R-A-K	17.98378	-0.89925	2.023704	5.99386	-2.63621	0.279925	-1.46997
80	T-S-K-Q-V-Q-L-Y-R-A-K-L-S-P-G	34.2292	-5.28146	-3.25664	-2.66791	-8.81643	-2.89614	-6.68902
81	V-Q-L-Y-R-A-K-L-S-P-G-D-V-F-V	35.77427	-5.17561	1.003032	0.577256	-8.31492	-4.88142	-7.93632
82	R-A-K-L-S-P-G-D-V-F-V-I-P-A-G	42.36209	-0.59944	3.197434	12.19635	-2.4858	0.839336	-3.4817
83	S-P-G-D-V-F-V-I-P-A-G-H-P-V-A	44.62468	0.802652	3.401417	32.10015	-0.21237	1.450046	-1.25612
84	V-F-V-I-P-A-G-H-P-V-A-I-N-A-S	38.6	0.054293	6.646819	18.18606	-2.00559	3.041365	-1.84458
85	P-A-G-H-P-V-A-I-N-A-S-S-D-L-N	22.94567	1.503215	3.683072	8.510971	-3.58964	1.717051	-3.20502
86	P-V-A-I-N-A-S-S-D-L-N-L-I-G-F	34.42912	2.346335	4.638937	10.59915	-4.10466	5.438116	-3.10712
87	N-A-S-S-D-L-N-L-I-G-F-G-I-N-A	36.27955	1.081879	-1.46738	18.36248	-5.15992	4.75053	-4.31898
88	D-L-N-L-I-G-F-G-I-N-A-E-N-N-E	41.70165	-0.98203	2.765379	26.95381	-2.48445	1.792561	-2.23743
89	I-G-F-G-I-N-A-E-N-N-E-R-N-F-L	32.7789	-1.20562	1.791902	9.935915	-2.94537	3.63183	-2.80602
90	I-N-A-E-N-N-E-R-N-F-L-A-G-E-E	26.43588	-11.4802	-5.37013	3.339923	-13.5591	-5.46546	-13.2407
91	N-N-E-R-N-F-L-A-G-E-E-D-N-V-I	41.71823	-2.64899	5.524388	29.45299	-3.04758	2.708808	-2.81142
92	N-F-L-A-G-E-E-D-N-V-I-S-Q-V-E	40.26949	-1.21274	0.408713	30.32234	-2.62698	0.240538	-2.22343
93	G-E-E-D-N-V-I-S-Q-V-E-R-P-V-K	25.52586	-2.37157	2.158329	11.21335	-3.60109	1.041618	-2.84263
94	N-V-I-S-Q-V-E-R-P-V-K-E-L-A-F	34.66043	0.274454	6.174538	11.31815	-2.74769	2.158549	-2.77759
95	Q-V-E-R-P-V-K-E-L-A-F-P-G-S-S	39.62622	33.04637	5.577837	27.17795	-5.19328	5.552355	9.540851
96	P-V-K-E-L-A-F-P-G-S-S-H-E-V-D	41.86569	23.22202	8.202726	29.73849	-3.3988	7.757944	9.21691
97	L-A-F-P-G-S-S-H-E-V-D-R-L-L-K	36.28509	-0.73579	7.223069	9.98607	-3.30081	-1.78469	-2.29882
98	G-S-S-H-E-V-D-R-L-L-K-N-Q-K-Q	31.0689	-2.42739	3.136829	-0.347	-4.16397	-1.69396	-1.14624
99	E-V-D-R-L-L-K-N-Q-K-Q-S-Y-F-A	22.86062	-1.81624	3.346059	1.752694	-6.87008	-6.0694	-4.10947
100	L-L-K-N-Q-K-Q-S-Y-F-A-N-A-Q-P	31.87528	2.058952	5.604967	28.93225	-3.26374	-1.92711	-2.18347
101	Q-K-Q-S-Y-F-A-N-A-Q-P-L-Q-R-E	40.19413	18.53059	7.956363	28.23146	-2.83293	1.982335	-1.45702
	m (blanks)	11374.69	10344.18	19821.69	10941.98	15028.41	16420.91	17118.34
	s (blanks)	1145.706	633.1946	3696.333	1513.151	2871.091	3507.765	2684.082

Table A44: Calculated Z-scores of Pis s 1 peptides after control subtraction.

The calculated Z-scores of every peptide of Pis s 1 for each pea-tolerant patient (patients 15-19) are listed. Identified candidate diagnostic peptides are highlighted in light blue.

	Patient No. →	15	16	17	18	19
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score
1	S-R-S-D-Q-E-N-P-F-I-F-K-S-N-R	-13.4603	-10.422	-7.52854	-8.78572	-12.6583
2	Q-E-N-P-F-I-F-K-S-N-R-F-Q-T-L	-16.9484	-8.90916	-1.64303	-7.68206	-15.4212
3	F-I-F-K-S-N-R-F-Q-T-L-Y-E-N-E	-4.20707	1.259707	-0.52012	-1.51547	-3.2172
4	S-N-R-F-Q-T-L-Y-E-N-E-N-G-H-I	-2.19337	0.085916	0.140929	-1.96043	-1.71911
5	Q-T-L-Y-E-N-E-N-G-H-I-R-L-L-Q	-2.0429	-1.08791	-0.2209	-2.47398	-1.68366
6	E-N-E-N-G-H-I-R-L-L-Q-K-F-D-K	-9.03846	-6.12228	0.165448	-5.56419	-7.96272
7	G-H-I-R-L-L-Q-K-F-D-K-R-S-K-I	-34.0497	-13.7884	1.634479	-17.6893	-27.9433
8	L-L-Q-K-F-D-K-R-S-K-I-F-E-N-L	-8.16534	-1.98529	-2.46481	-5.54022	-6.46899
9	F-D-K-R-S-K-I-F-E-N-L-Q-N-Y-R	-17.7182	-8.83819	-5.28659	-11.1148	-16.9958
10	S-K-I-F-E-N-L-Q-N-Y-R-L-L-E-Y	-2.97589	-1.83706	-1.12713	-3.19246	-3.24367
11	E-N-L-Q-N-Y-R-L-L-E-Y-K-S-K-P	-6.62683	-3.72966	-0.39366	-3.07698	-4.52322
12	N-Y-R-L-L-E-Y-K-S-K-P-H-T-L-F	-10.8086	-3.08973	-0.37147	-0.76911	-8.49749
13	L-E-Y-K-S-K-P-H-T-L-F-L-P-Q-Y	-10.1416	2.571167	0.237506	-1.92509	-9.67602
14	S-K-P-H-T-L-F-L-P-Q-Y-T-D-A-D	-3.57086	-0.66793	0.593583	-2.0426	-2.87127
15	T-L-F-L-P-Q-Y-T-D-A-D-F-I-L-V	-4.48562	0.050592	-2.26893	-3.20423	-4.78392
16	P-Q-Y-T-D-A-D-F-I-L-V-V-L-S-G	-5.06597	-0.47533	1.631879	-4.30273	-5.82983
17	D-A-D-F-I-L-V-V-L-S-G-K-A-T-L	-18.219	-12.4306	-10.6441	-14.7891	-17.5906
18	I-L-V-V-L-S-G-K-A-T-L-T-V-L-K	-13.7903	-4.1188	-4.10497	-6.41761	-12.3622
19	L-S-G-K-A-T-L-T-V-L-K-S-N-D-R	-7.71387	-3.57051	-1.72013	-1.47568	-4.98055
20	A-T-L-T-V-L-K-S-N-D-R-N-S-F-N	-5.39905	-0.56084	-2.27481	-0.85004	-0.88524
21	V-L-K-S-N-D-R-N-S-F-N-L-E-R-G	-4.13897	-0.48813	-1.56806	-2.04585	-1.42867
22	N-D-R-N-S-F-N-L-E-R-G-D-A-I-K	-6.43281	-1.80049	-4.07451	-5.24309	-4.49879
23	S-F-N-L-E-R-G-D-A-I-K-L-P-A-G	-5.64984	-1.76849	-2.18166	-4.475	-3.55776
24	E-R-G-D-A-I-K-L-P-A-G-T-I-A-Y	-4.66715	-0.80011	-0.89253	-3.17447	-2.29159
25	A-I-K-L-P-A-G-T-I-A-Y-L-A-N-R	-10.2625	-5.81982	-6.3322	-7.1863	-9.84547
26	P-A-G-T-I-A-Y-L-A-N-R-D-D-N-E	-5.35885	-1.26187	-2.34948	-3.55645	-5.58816
27	I-A-Y-L-A-N-R-D-D-N-E-D-L-R-V	-7.97583	-1.99836	-2.72029	-5.15946	-8.62149
28	A-N-R-D-D-N-E-D-L-R-V-L-D-L-A	-2.21238	0.472777	-0.44397	-1.7264	-2.95764
29	D-N-E-D-L-R-V-L-D-L-A-I-P-V-N	-3.01174	1.935923	0.513233	-2.61532	-3.04264
30	L-R-V-L-D-L-A-I-P-V-N-K-P-G-Q	-1.57763	3.600266	0.125761	-0.8336	-1.53395
31	D-L-A-I-P-V-N-K-P-G-Q-L-Q-S-F	-5.05828	-1.58129	-2.97782	-5.45859	-4.36359
32	P-V-N-K-P-G-Q-L-Q-S-F-L-L-S-G	-3.33606	1.974875	-0.04909	-2.78614	-2.70182
33	P-G-Q-L-Q-S-F-L-L-S-G-T-Q-N-Q	-6.36312	0.878546	-3.95481	-5.80052	-5.88136
34	Q-S-F-L-L-S-G-T-Q-N-Q-P-S-L-L	-2.84126	3.166369	-1.11093	-1.4832	-2.62268
35	L-S-G-T-Q-N-Q-P-S-L-L-S-G-F-S	-4.91525	-2.32246	-3.66383	-5.34223	-5.11906
36	Q-N-Q-P-S-L-L-S-G-F-S-K-N-I-L	-3.07291	6.333727	-1.32427	-2.35792	-3.2983
37	S-L-L-S-G-F-S-K-N-I-L-E-A-A-F	-1.83071	5.118468	-1.1236	-1.96441	-2.19508
38	G-F-S-K-N-I-L-E-A-A-F-N-T-N-Y	-3.34711	-0.27968	-1.87905	-2.4637	-3.45209
39	N-I-L-E-A-A-F-N-T-N-Y-E-E-I-E	-2.91487	-2.66201	-2.64225	-2.88285	-2.60401
40	A-A-F-N-T-N-Y-E-E-I-E-K-V-L-L	-3.1865	-2.04753	-3.19696	-2.64777	-2.81491

41	T-N-Y-E-E-I-E-K-V-L-L-E-Q-Q-E	-3.36837	-3.10694	-3.52619	-2.58193	-2.60948
42	E-I-E-K-V-L-L-E-Q-Q-E-Q-E-P-Q	-3.30013	-1.69357	-2.303	-2.52566	-2.14668
43	V-L-L-E-Q-Q-E-Q-E-P-Q-H-R-R-S	-3.15423	-2.36111	-1.46756	-1.62396	-1.08916
44	Q-Q-E-Q-E-P-Q-H-R-R-S-L-K-D-R	-3.38302	-0.83284	-1.52812	-1.78652	-1.75911
45	E-P-Q-H-R-R-S-L-K-D-R-R-Q-E-I	-15.4728	-5.12003	-7.37737	-6.03377	-9.77115
46	R-R-S-L-K-D-R-R-Q-E-I-N-E-E-N	-3.46304	0.856794	-1.73739	-2.2206	-2.51251
47	K-D-R-R-Q-E-I-N-E-E-N-V-I-V-K	-6.46214	-3.06195	-4.1582	-4.58095	-4.67381
48	Q-E-I-N-E-E-N-V-I-V-K-V-S-R-E	-4.35652	3.650018	-0.28099	-1.5872	-2.33384
49	E-E-N-V-I-V-K-V-S-R-E-Q-I-E-E	-3.74768	1.076509	-2.17483	-2.48467	-4.08036
50	I-V-K-V-S-R-E-Q-I-E-E-L-S-K-N	-3.83605	0.397113	-2.71479	-3.30659	-3.1948
51	S-R-E-Q-I-E-E-L-S-K-N-A-K-S-S	-4.02411	-0.6716	-2.56331	-3.85023	-4.39627
52	I-E-E-L-S-K-N-A-K-S-S-S-K-K-S	-17.3006	-9.29332	-0.54867	-13.1742	-15.2045
53	S-K-N-A-K-S-S-S-K-K-S-V-S-S-E	-6.13419	-0.45986	-2.15514	-5.85461	-6.0273
54	K-S-S-S-K-K-S-V-S-S-E-S-G-P-F	-3.79553	0.704617	-1.97862	-3.77091	-2.65291
55	K-K-S-V-S-S-E-S-G-P-F-N-L-R-S	-2.42465	3.158841	-1.36425	-1.93768	-1.74967
56	S-S-E-S-G-P-F-N-L-R-S-R-N-P-I	-5.95837	-2.84435	-4.0608	-4.3827	-4.66199
57	G-P-F-N-L-R-S-R-N-P-I-Y-S-N-K	-38.3891	-19.8819	-0.01358	-12.6886	-21.9217
58	L-R-S-R-N-P-I-Y-S-N-K-F-G-K-F	-42.4633	-11.6145	-7.83901	-23.2642	-30.9289
59	N-P-I-Y-S-N-K-F-G-K-F-F-E-I-T	-3.6033	0.747322	-1.97963	-2.36429	-3.64132
60	S-N-K-F-G-K-F-F-E-I-T-P-E-K-N	-1.33034	0.434152	-0.68787	-1.70076	-1.90578
61	G-K-F-F-E-I-T-P-E-K-N-Q-Q-L-Q	-1.37353	-0.48238	-1.49369	-1.69566	-1.88514
62	E-I-T-P-E-K-N-Q-Q-L-Q-D-L-D-I	-1.85371	0.567526	-2.56902	-2.72708	-2.67114
63	E-K-N-Q-Q-L-Q-D-L-D-I-F-V-N-S	-1.34824	-0.44501	-2.46839	-1.66056	-1.59287
64	Q-L-Q-D-L-D-I-F-V-N-S-V-D-I-K	-1.20803	0.402305	-0.80799	-1.07828	-0.11545
65	L-D-I-F-V-N-S-V-D-I-K-E-G-S-L	-1.09874	-1.066	-1.46389	-1.39026	-0.81339
66	V-N-S-V-D-I-K-E-G-S-L-L-L-P-N	-3.08318	0.17098	-2.2453	-1.32905	-1.45367
67	D-I-K-E-G-S-L-L-L-P-N-Y-N-S-R	-4.26151	-1.8026	-1.8897	0.00886	-3.23673
68	G-S-L-L-L-P-N-Y-N-S-R-A-I-V-I	-5.37453	-0.49335	-1.69539	-2.12186	-5.20999
69	L-P-N-Y-N-S-R-A-I-V-I-V-T-V-T	-5.58824	10.90429	-0.73141	-0.45558	-4.81024
70	N-S-R-A-I-V-I-V-T-V-T-E-G-K-G	-8.37323	1.487374	-3.16822	-4.20666	-6.51673
71	I-V-I-V-T-V-T-E-G-K-G-D-F-E-L	-3.08293	2.872502	-1.75592	-1.69986	-1.73316
72	T-V-T-E-G-K-G-D-F-E-L-V-G-Q-R	-4.59664	2.592549	-1.96595	-2.74241	-2.96915
73	G-K-G-D-F-E-L-V-G-Q-R-N-E-N-Q	-5.15821	-1.70502	-4.29219	-3.4089	-5.35218
74	F-E-L-V-G-Q-R-N-E-N-Q-G-K-E-N	-2.12256	-0.15217	-1.2786	-1.25687	-2.58318
75	G-Q-R-N-E-N-Q-G-K-E-N-D-K-E-E	-3.72655	-2.52896	-3.24724	-3.39677	-4.12105
76	E-N-Q-G-K-E-N-D-K-E-E-E-Q-E-E	-2.8132	-2.48367	-1.63009	-3.03207	-2.99558
77	K-E-N-D-K-E-E-E-Q-E-E-E-T-S-K	-1.74724	-0.88303	-0.8686	-2.73222	-2.92119
78	K-E-E-E-Q-E-E-E-T-S-K-Q-V-Q-L	-2.26655	0.164954	-1.16767	-2.4701	-2.47922
79	Q-E-E-E-T-S-K-Q-V-Q-L-Y-R-A-K	-2.76508	1.664128	-1.39374	-0.85452	-3.39084
80	T-S-K-Q-V-Q-L-Y-R-A-K-L-S-P-G	-7.65786	1.434236	6.686576	9.847755	-7.44623
81	V-Q-L-Y-R-A-K-L-S-P-G-D-V-F-V	-6.56392	8.931629	0.69736	1.456627	-4.44008
82	R-A-K-L-S-P-G-D-V-F-V-I-P-A-G	-1.55695	49.89676	-1.41812	-1.33788	8.920229
83	S-P-G-D-V-F-V-I-P-A-G-H-P-V-A	0.260205	15.19058	-0.16898	-1.00629	-0.08154
84	V-F-V-I-P-A-G-H-P-V-A-I-N-A-S	-0.34396	11.61097	-1.49301	-1.48172	-0.70142
85	P-A-G-H-P-V-A-I-N-A-S-S-D-L-N	-1.60097	5.151142	-2.40592	-2.5114	-1.71168

86	P-V-A-I-N-A-S-S-D-L-N-L-I-G-F	-1.2487	29.55598	-2.00901	-2.31034	-2.36744
87	N-A-S-S-D-L-N-L-I-G-F-G-I-N-A	-2.8855	5.178131	-3.62042	-1.66584	0.74321
88	D-L-N-L-I-G-F-G-I-N-A-E-N-N-E	-0.95704	11.27963	-2.00867	-1.54164	11.95395
89	I-G-F-G-I-N-A-E-N-N-E-R-N-F-L	-1.95184	8.479497	-2.54269	-2.03195	-1.28217
90	I-N-A-E-N-N-E-R-N-F-L-A-G-E-E	-11.4088	-3.44133	-13.3101	-13.5696	-12.8608
91	N-N-E-R-N-F-L-A-G-E-E-D-N-V-I	-1.40201	32.40804	-3.27134	-3.43653	-2.3998
92	N-F-L-A-G-E-E-D-N-V-I-S-Q-V-E	-2.01481	5.250574	-2.1614	-2.02444	-1.41137
93	G-E-E-D-N-V-I-S-Q-V-E-R-P-V-K	-2.17424	1.992644	-1.65407	-2.02686	-1.77432
94	N-V-I-S-Q-V-E-R-P-V-K-E-L-A-F	-2.85441	2.784612	-2.09036	-2.08895	-0.28559
95	Q-V-E-R-P-V-K-E-L-A-F-P-G-S-S	-6.15842	28.97455	-5.02417	-4.01177	-3.46389
96	P-V-K-E-L-A-F-P-G-S-S-H-E-V-D	-4.39766	37.95628	-2.58731	-2.60492	-1.87354
97	L-A-F-P-G-S-S-H-E-V-D-R-L-L-K	-3.22764	0.250596	-2.35524	-0.90021	-3.54301
98	G-S-S-H-E-V-D-R-L-L-K-N-Q-K-Q	-4.03293	-2.79565	-3.46959	-2.64969	-4.51543
99	E-V-D-R-L-L-K-N-Q-K-Q-S-Y-F-A	-6.82711	-5.52933	-3.11373	-3.62955	-6.78223
100	L-L-K-N-Q-K-Q-S-Y-F-A-N-A-Q-P	-3.17756	-0.98784	-2.02133	-2.08102	-3.63068
101	Q-K-Q-S-Y-F-A-N-A-Q-P-L-Q-R-E	-1.89472	8.403089	-0.82905	-1.02467	-3.24907
	m (blanks)	17725.34	10699.67	10097.17	10987.35	10346.84
	s (blanks)	2939.751	954.8014	570.9965	1183.068	1124.607

Table A45: Calculated Z-scores of Pis s 1 peptides of atopic controls.

The calculated Z-scores of every peptide of Pis s 1 for each atopic control serum (A-E) are listed.

	Control No. →	Serum A	Serum B	Serum C	Serum D	Serum E
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score
1	S-R-S-D-Q-E-N-P-F-I-F-K-S-N-R	1.966491	2.267412	2.080794	1.632326	4.039627
2	Q-E-N-P-F-I-F-K-S-N-R-F-Q-T-L	7.428312	6.18019	5.673549	4.106311	17.77417
3	F-I-F-K-S-N-R-F-Q-T-L-Y-E-N-E	1.241756	1.721836	1.584658	1.55256	3.990945
4	S-N-R-F-Q-T-L-Y-E-N-E-N-G-H-I	0.52381	0.470853	0.784075	0.444564	2.026911
5	Q-T-L-Y-E-N-E-N-G-H-I-R-L-L-Q	0.688445	0.29415	0.579701	0.31993	2.227726
6	E-N-E-N-G-H-I-R-L-L-Q-K-F-D-K	1.635523	3.045937	3.1675	2.137093	6.317055
7	G-H-I-R-L-L-Q-K-F-D-K-R-S-K-I	11.14534	10.03297	14.59413	14.19419	26.27383
8	L-L-Q-K-F-D-K-R-S-K-I-F-E-N-L	1.391116	2.018997	2.200599	2.552125	4.521888
9	F-D-K-R-S-K-I-F-E-N-L-Q-N-Y-R	8.831956	4.665793	4.646041	4.632266	9.635071
10	S-K-I-F-E-N-L-Q-N-Y-R-L-L-E-Y	0.209814	-0.07144	0.841864	0.379754	0.453858
11	E-N-L-Q-N-Y-R-L-L-E-Y-K-S-K-P	1.396207	1.18329	0.581111	2.604471	0.969588
12	N-Y-R-L-L-E-Y-K-S-K-P-H-T-L-F	2.674253	2.213042	0.992678	2.815102	5.389045
13	L-E-Y-K-S-K-P-H-T-L-F-L-P-Q-Y	7.243309	3.307008	1.397198	6.149062	3.513248
14	S-K-P-H-T-L-F-L-P-Q-Y-T-D-A-D	0.724088	0.844414	0.101889	0.213991	2.218598
15	T-L-F-L-P-Q-Y-T-D-A-D-F-I-L-V	1.937637	1.412488	0.396469	0.877044	2.676518
16	P-Q-Y-T-D-A-D-F-I-L-V-V-L-S-G	3.203802	2.419274	1.308401	1.628587	4.658808
17	D-A-D-F-I-L-V-V-L-S-G-K-A-T-L	9.327559	3.699785	2.998363	4.378013	6.266851
18	I-L-V-V-L-S-G-K-A-T-L-T-V-L-K	4.709282	3.599013	3.721424	4.451547	4.855059
19	L-S-G-K-A-T-L-T-V-L-K-S-N-D-R	2.143007	3.165458	1.677684	1.647282	3.108575
20	A-T-L-T-V-L-K-S-N-D-R-N-S-F-N	1.428456	2.573948	1.354913	1.440389	2.331176
21	V-L-K-S-N-D-R-N-S-F-N-L-E-R-G	1.372446	2.063056	0.605072	0.685107	2.481788

22	N-D-R-N-S-F-N-L-E-R-G-D-A-I-K	1.382629	1.969314	1.653722	0.651456	1.562906
23	S-F-N-L-E-R-G-D-A-I-K-L-P-A-G	0.483075	0.797543	0.358414	0.140457	0.78855
24	E-R-G-D-A-I-K-L-P-A-G-T-I-A-Y	-0.16528	-0.11831	0.358414	0.200281	0.237829
25	A-I-K-L-P-A-G-T-I-A-Y-L-A-N-R	1.139919	2.285692	1.36337	2.05982	4.605561
26	P-A-G-T-I-A-Y-L-A-N-R-D-D-N-E	2.585995	2.073367	1.340819	1.637311	3.499556
27	I-A-Y-L-A-N-R-D-D-N-E-D-L-R-V	4.55483	3.089058	2.09066	2.931012	4.289125
28	A-N-R-D-D-N-E-D-L-R-V-L-D-L-A	1.105974	0.673804	1.073018	1.066487	2.664347
29	D-N-E-D-L-R-V-L-D-L-A-I-P-V-N	0.724088	1.319684	1.614257	0.944346	2.411807
30	L-R-V-L-D-L-A-I-P-V-N-K-P-G-Q	0.50514	0.948936	1.625533	1.451606	2.273366
31	D-L-A-I-P-V-N-K-P-G-Q-L-Q-S-F	0.937944	0.46851	3.250659	0.645224	1.185617
32	P-V-N-K-P-G-Q-L-Q-S-F-L-L-S-G	0.578122	-0.11784	1.228061	1.379319	2.153181
33	P-G-Q-L-Q-S-F-L-L-S-G-T-Q-N-Q	0.605279	0.191972	1.332362	1.462823	1.903683
34	Q-S-F-L-L-S-G-T-Q-N-Q-P-S-L-L	0.391422	0.325085	1.347866	1.582472	1.806318
35	L-S-G-T-Q-N-Q-P-S-L-L-S-G-F-S	0.124951	-0.0991	0.705145	0.802263	1.337749
36	Q-N-Q-P-S-L-L-S-G-F-S-K-N-I-L	0.111373	0.363519	0.519094	0.76238	1.065432
37	S-L-L-S-G-F-S-K-N-I-L-E-A-A-F	0.252246	-0.02785	0.310491	0.362305	0.637939
38	G-F-S-K-N-I-L-E-A-A-F-N-T-N-Y	0.4678	0.089793	0.09766	0.218976	0.936119
39	N-I-L-E-A-A-F-N-T-N-Y-E-E-I-E	0.454222	0.120259	0.0441	-0.2484	0.205882
40	A-A-F-N-T-N-Y-E-E-I-E-K-V-L-L	0.678261	0.447886	0.309082	0.086864	1.328621
41	T-N-Y-E-E-I-E-K-V-L-L-E-Q-Q-E	1.428456	0.655055	0.361233	0.185325	1.26929
42	E-I-E-K-V-L-L-E-Q-Q-E-Q-E-P-Q	0.260733	0.476946	0.39506	0.007098	1.363612
43	V-L-L-E-Q-Q-E-Q-E-P-Q-H-R-R-S	0.610371	0.657868	0.330224	0.186571	0.567958
44	Q-Q-E-Q-E-P-Q-H-R-R-S-L-K-D-R	2.871137	2.09399	1.061742	1.995011	1.864129
45	E-P-Q-H-R-R-S-L-K-D-R-R-Q-E-I	15.80944	6.366736	9.498868	12.49418	6.342918
46	R-R-S-L-K-D-R-R-Q-E-I-N-E-E-N	0.937944	1.449985	1.178729	0.18034	1.273854
47	K-D-R-R-Q-E-I-N-E-E-N-V-I-V-K	0.265824	0.506006	0.213238	0.280047	0.619683
48	Q-E-I-N-E-E-N-V-I-V-K-V-S-R-E	1.000743	0.537878	0.481038	0.787307	0.698792
49	E-E-N-V-I-V-K-V-S-R-E-Q-I-E-E	1.027899	1.853074	1.184367	1.224772	3.421968
50	I-V-K-V-S-R-E-Q-I-E-E-L-S-K-N	0.520415	1.323902	0.82636	1.206077	4.419959
51	S-R-E-Q-I-E-E-L-S-K-N-A-K-S-S	0.922669	1.373585	1.130807	1.228511	3.035551
52	I-E-E-L-S-K-N-A-K-S-S-S-K-K-S	9.054298	6.876691	8.297994	11.1095	18.1682
53	S-K-N-A-K-S-S-S-K-K-S-V-S-S-E	1.160287	1.381085	1.784804	1.768177	4.973723
54	K-S-S-S-K-K-S-V-S-S-E-S-G-P-F	0.863264	0.207908	1.563516	2.095964	2.738892
55	K-K-S-V-S-S-E-S-G-P-F-N-L-R-S	0.501745	0.259934	1.285849	1.075212	2.471139
56	S-S-E-S-G-P-F-N-L-R-S-R-N-P-I	2.285578	0.743173	5.156271	3.212685	6.423548
57	G-P-F-N-L-R-S-R-N-P-I-Y-S-N-K	18.30952	8.158374	22.91708	38.74958	42.80686
58	L-R-S-R-N-P-I-Y-S-N-K-F-G-K-F	22.3049	12.63524	29.76855	44.96009	49.68707
59	N-P-I-Y-S-N-K-F-G-K-F-F-E-I-T	1.146708	1.336558	1.71292	2.537169	4.921998
60	S-N-K-F-G-K-F-F-E-I-T-P-E-K-N	0.57982	0.891285	0.499361	0.708788	2.058859
61	G-K-F-F-E-I-T-P-E-K-N-Q-Q-L-Q	0.369358	0.055109	0.2076	0.004606	0.913299
62	E-I-T-P-E-K-N-Q-Q-L-Q-D-L-D-I	0.72239	-0.01192	0.734744	0.192803	1.412294
63	E-K-N-Q-Q-L-Q-D-L-D-I-F-V-N-S	1.009229	-0.2158	0.654404	0.065676	1.287546
64	Q-L-Q-D-L-D-I-F-V-N-S-V-D-I-K	1.229875	0.389298	0.603662	0.549256	1.801754
65	L-D-I-F-V-N-S-V-D-I-K-E-G-S-L	0.810649	-0.02551	0.221694	0.160398	1.582683
66	V-N-S-V-D-I-K-E-G-S-L-L-L-P-N	0.732574	0.396328	0.600844	0.427115	2.159266

67	D-I-K-E-G-S-L-L-L-P-N-Y-N-S-R	2.331404	1.438267	0.641718	1.035329	3.466087
68	G-S-L-L-L-P-N-Y-N-S-R-A-I-V-I	3.962483	2.643317	1.33659	2.729105	3.449352
69	L-P-N-Y-N-S-R-A-I-V-I-V-T-V-T	6.936103	3.66182	3.610076	4.513864	6.913416
70	N-S-R-A-I-V-I-V-T-V-T-E-G-K-G	5.982236	4.549554	4.416296	3.525516	5.986927
71	I-V-I-V-T-V-T-E-G-K-G-D-F-E-L	0.751244	0.545846	0.170953	0.273815	0.534488
72	T-V-T-E-G-K-G-D-F-E-L-V-G-Q-R	0.814043	0.691615	0.209009	0.746178	-1.06138
73	G-K-G-D-F-E-L-V-G-Q-R-N-E-N-Q	1.145011	1.736366	1.050467	1.384304	2.279451
74	F-E-L-V-G-Q-R-N-E-N-Q-G-K-E-N	1.402997	1.091892	1.002544	0.956809	2.623271
75	G-Q-R-N-E-N-Q-G-K-E-N-D-K-E-E	1.090699	0.786763	0.624805	0.777336	1.889991
76	E-N-Q-G-K-E-N-D-K-E-E-E-Q-E-E	0.50514	0.29415	0.788304	0.402188	1.144541
77	K-E-N-D-K-E-E-E-Q-E-E-E-T-S-K	0.267522	0.328366	0.974355	0.298742	1.471626
78	K-E-E-E-Q-E-E-E-T-S-K-Q-V-Q-L	0.342202	-0.13519	0.63608	0.298742	1.576598
79	Q-E-E-E-T-S-K-Q-V-Q-L-Y-R-A-K	1.627036	0.398203	1.562106	1.619862	3.610613
80	T-S-K-Q-V-Q-L-Y-R-A-K-L-S-P-G	6.275864	3.460275	5.315542	7.984921	8.964165
81	V-Q-L-Y-R-A-K-L-S-P-G-D-V-F-V	4.59896	3.780872	3.253478	7.28697	9.254739
82	R-A-K-L-S-P-G-D-V-F-V-I-P-A-G	0.912485	0.318523	1.126578	0.833422	3.148129
83	S-P-G-D-V-F-V-I-P-A-G-H-P-V-A	0.186053	-1.07823	0.352776	0.134225	-0.22618
84	V-F-V-I-P-A-G-H-P-V-A-I-N-A-S	1.995344	-0.34048	1.789032	0.798524	1.389475
85	P-A-G-H-P-V-A-I-N-A-S-S-D-L-N	1.924059	0.107604	0.435935	0.665166	1.217565
86	P-V-A-I-N-A-S-S-D-L-N-L-I-G-F	2.221082	0.387892	0.396469	0.569198	2.250546
87	N-A-S-S-D-L-N-L-I-G-F-G-I-N-A	3.891197	0.373362	0.328815	0.506881	1.839788
88	D-L-N-L-I-G-F-G-I-N-A-E-N-N-E	1.672863	0.023237	0.059605	0.160398	1.622238
89	I-G-F-G-I-N-A-E-N-N-E-R-N-F-L	1.233269	0.41742	0.524732	0.80351	2.87429
90	I-N-A-E-N-N-E-R-N-F-L-A-G-E-E	0.724088	0.56647	0.311901	0.677629	1.062389
91	N-N-E-R-N-F-L-A-G-E-E-D-N-V-I	0.763125	0.664898	0.661451	0.400942	2.03756
92	N-F-L-A-G-E-E-D-N-V-I-S-Q-V-E	1.03978	0.622715	1.075837	1.118834	1.768285
93	G-E-E-D-N-V-I-S-Q-V-E-R-P-V-K	2.005528	1.072206	2.014548	1.221033	2.080157
94	N-V-I-S-Q-V-E-R-P-V-K-E-L-A-F	2.025895	1.132201	2.368327	1.272133	3.078148
95	Q-V-E-R-P-V-K-E-L-A-F-P-G-S-S	0.934549	0.612872	2.664317	1.390536	1.564427
96	P-V-K-E-L-A-F-P-G-S-S-H-E-V-D	0.564544	-0.13519	1.057514	0.686354	-0.51979
97	L-A-F-P-G-S-S-H-E-V-D-R-L-L-K	1.689835	0.711769	1.1745	0.806002	1.445764
98	G-S-S-H-E-V-D-R-L-L-K-N-Q-K-Q	1.442034	1.986656	1.867963	0.946839	3.009689
99	E-V-D-R-L-L-K-N-Q-K-Q-S-Y-F-A	2.830402	2.117426	3.62558	3.086804	6.046259
100	L-L-K-N-Q-K-Q-S-Y-F-A-N-A-Q-P	1.184048	0.540691	1.151949	1.966345	1.620716
101	Q-K-Q-S-Y-F-A-N-A-Q-P-L-Q-R-E	2.789668	0.087918	0.69105	0.523083	2.273366
	m (blanks)	9352.381	12190.42	9346.712	9851.305	10695.67
	s (blanks)	589.1808	2133.523	709.4833	802.3493	657.3206

Table A46: Calculated Z-scores of Pis s 1 peptides of non-atopic controls and derived maximum Z-score of all controls (A-J).

The calculated Z-scores of every peptide of Pis s 1 for each non-atopic control serum (F-J) and the derived maximum Z-score of atopic and non-atopic controls are listed.

	Control No. →	Serum F	Serum G	Serum H	Serum I	Serum J	Max. (A-J)
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score	Z-score	Z-score
1	S-R-S-D-Q-E-N-P-F-I-F-K-S-N-R	1.475394	13.13598	0.644663	2.121503	3.121124	13.13598
2	Q-E-N-P-F-I-F-K-S-N-R-F-Q-T-L	4.501443	15.07625	3.140877	3.693318	14.78129	17.77417
3	F-I-F-K-S-N-R-F-Q-T-L-Y-E-N-E	0.790414	3.964828	0.68646	1.165285	2.410617	3.990945
4	S-N-R-F-Q-T-L-Y-E-N-E-N-G-H-I	0.504284	1.225024	-0.0566	0.454314	1.518057	2.026911
5	Q-T-L-Y-E-N-E-N-G-H-I-R-L-L-Q	0.568446	0.895512	0.287066	0.332929	2.376482	2.376482
6	E-N-E-N-G-H-I-R-L-L-Q-K-F-D-K	1.504874	10.38948	1.624572	1.233409	7.611736	10.38948
7	G-H-I-R-L-L-Q-K-F-D-K-R-S-K-I	10.3888	37.02395	18.14603	13.26169	25.91806	37.02395
8	L-L-Q-K-F-D-K-R-S-K-I-F-E-N-L	1.660945	8.12304	1.640827	1.227216	6.266575	8.12304
9	F-D-K-R-S-K-I-F-E-N-L-Q-N-Y-R	3.04998	18.24258	3.702816	3.485229	12.10614	18.24258
10	S-K-I-F-E-N-L-Q-N-Y-R-L-L-E-Y	0.190407	2.865896	0.472831	0.183056	1.549663	2.865896
11	E-N-L-Q-N-Y-R-L-L-E-Y-K-S-K-P	0.356883	7.029126	2.648601	2.091776	3.318347	7.029126
12	N-Y-R-L-L-E-Y-K-S-K-P-H-T-L-F	1.811814	10.77754	3.377728	1.198728	7.387964	10.77754
13	L-E-Y-K-S-K-P-H-T-L-F-L-P-Q-Y	2.002568	11.36297	4.919575	6.164374	8.642099	11.36297
14	S-K-P-H-T-L-F-L-P-Q-Y-T-D-A-D	0.407173	3.369364	0.881513	0.528632	2.824026	3.369364
15	T-L-F-L-P-Q-Y-T-D-A-D-F-I-L-V	1.272501	5.324694	2.985299	0.706994	3.544648	5.324694
16	P-Q-Y-T-D-A-D-F-I-L-V-V-L-S-G	2.092742	6.938803	4.601453	3.945997	6.552295	6.938803
17	D-A-D-F-I-L-V-V-L-S-G-K-A-T-L	2.685813	20.62109	4.866168	5.350598	10.16678	20.62109
18	I-L-V-V-L-S-G-K-A-T-L-T-V-L-K	2.382341	14.76849	4.761675	3.368798	9.276751	14.76849
19	L-S-G-K-A-T-L-T-V-L-K-S-N-D-R	1.520481	7.46067	2.527854	1.053809	3.281684	7.46067
20	A-T-L-T-V-L-K-S-N-D-R-N-S-F-N	1.912393	3.180354	4.130075	0.321782	4.645808	4.645808
21	V-L-K-S-N-D-R-N-S-F-N-L-E-R-G	2.174246	2.544747	3.424169	0.576938	2.282928	3.424169
22	N-D-R-N-S-F-N-L-E-R-G-D-A-I-K	2.219334	6.107495	3.7748	0.454314	4.338596	6.107495
23	S-F-N-L-E-R-G-D-A-I-K-L-P-A-G	2.399683	1.37389	4.766319	0.786266	2.133746	4.766319
24	E-R-G-D-A-I-K-L-P-A-G-T-I-A-Y	2.332052	0.726574	3.027097	0.064148	2.065477	3.027097
25	A-I-K-L-P-A-G-T-I-A-Y-L-A-N-R	0.733188	10.38446	0.284744	1.91713	3.719114	10.38446
26	P-A-G-T-I-A-Y-L-A-N-R-D-D-N-E	0.781744	5.585627	-0.26791	1.334977	4.398016	5.585627
27	I-A-Y-L-A-N-R-D-D-N-E-D-L-R-V	1.726842	8.872388	1.269297	3.578126	5.418264	8.872388
28	A-N-R-D-D-N-E-D-L-R-V-L-D-L-A	0.911803	1.526102	-0.00551	0.838288	2.363839	2.664347
29	D-N-E-D-L-R-V-L-D-L-A-I-P-V-N	0.663823	0.999216	0.136132	0.71938	3.486492	3.486492
30	L-R-V-L-D-L-A-I-P-V-N-K-P-G-Q	0.653418	1.962664	0.303321	0.923753	2.576233	2.576233
31	D-L-A-I-P-V-N-K-P-G-Q-L-Q-S-F	0.434919	2.670195	1.285552	0.750346	5.166679	5.166679
32	P-V-N-K-P-G-Q-L-Q-S-F-L-L-S-G	0.721049	2.347374	0.391559	0.576938	3.167902	3.167902
33	P-G-Q-L-Q-S-F-L-L-S-G-T-Q-N-Q	0.533764	6.30654	1.494537	1.135558	3.539591	6.30654
34	Q-S-F-L-L-S-G-T-Q-N-Q-P-S-L-L	0.701974	2.800662	1.76854	0.580654	1.438409	2.800662
35	L-S-G-T-Q-N-Q-P-S-L-L-S-G-F-S	0.584053	0.537565	0.58429	0.132272	5.213456	5.213456
36	Q-N-Q-P-S-L-L-S-G-F-S-K-N-I-L	0.707176	3.366019	0.84436	0.127318	1.396689	3.366019
37	S-L-L-S-G-F-S-K-N-I-L-E-A-A-F	0.563244	0.763372	1.501503	0.191726	2.188109	2.188109
38	G-F-S-K-N-I-L-E-A-A-F-N-T-N-Y	0.486942	1.39229	0.909378	0.424587	3.745663	3.745663
39	N-I-L-E-A-A-F-N-T-N-Y-E-E-I-E	0.556307	0.624542	1.071922	-0.07334	2.901145	2.901145

40	A-A-F-N-T-N-Y-E-E-I-E-K-V-L-L	0.851109	1.863977	0.656274	1.036468	3.666016	3.666016
41	T-N-Y-E-E-I-E-K-V-L-L-E-Q-Q-E	1.152847	3.839379	1.559555	0.526155	2.995964	3.839379
42	E-I-E-K-V-L-L-E-Q-Q-E-Q-E-P-Q	1.322791	-0.01107	0.588934	-0.29505	3.068026	3.068026
43	V-L-L-E-Q-Q-E-Q-E-P-Q-H-R-R-S	1.501406	0.201362	1.05799	-0.14023	2.563591	2.563591
44	Q-Q-E-Q-E-P-Q-H-R-R-S-L-K-D-R	2.233207	3.454669	3.468288	1.244557	2.989643	3.468288
45	E-P-Q-H-R-R-S-L-K-D-R-R-Q-E-I	13.03334	19.99887	13.39974	18.69182	17.6347	19.99887
46	R-R-S-L-K-D-R-R-Q-E-I-N-E-E-N	2.430897	1.688349	2.776314	0.545973	3.336047	3.336047
47	K-D-R-R-Q-E-I-N-E-E-N-V-I-V-K	2.481187	0.835297	3.071216	0.222692	5.403093	5.403093
48	Q-E-I-N-E-E-N-V-I-V-K-V-S-R-E	2.918186	1.303639	2.799535	0.524916	2.277871	2.918186
49	E-E-N-V-I-V-K-V-S-R-E-Q-I-E-E	0.603129	4.160528	0.078081	1.711519	3.394202	4.160528
50	I-V-K-V-S-R-E-Q-I-E-E-L-S-K-N	0.59099	1.830524	-0.12394	1.554213	4.422036	4.422036
51	S-R-E-Q-I-E-E-L-S-K-N-A-K-S-S	0.825097	1.29193	0.222048	1.271807	4.815218	4.815218
52	I-E-E-L-S-K-N-A-K-S-S-S-K-K-S	6.324008	20.19122	4.164906	7.109445	15.29331	20.19122
53	S-K-N-A-K-S-S-S-K-K-S-V-S-S-E	1.090418	3.197081	0.331185	1.585179	6.920192	6.920192
54	K-S-S-S-K-K-S-V-S-S-E-S-G-P-F	0.544169	0.616179	0.303321	1.076104	3.887259	3.887259
55	K-K-S-V-S-S-E-S-G-P-F-N-L-R-S	0.714113	-0.0211	0.379949	0.31435	2.518078	2.518078
56	S-S-E-S-G-P-F-N-L-R-S-R-N-P-I	3.186976	2.466132	1.882321	1.574031	6.442306	6.442306
57	G-P-F-N-L-R-S-R-N-P-I-Y-S-N-K	14.77093	33.217	44.98904	22.7285	30.68491	44.98904
58	L-R-S-R-N-P-I-Y-S-N-K-F-G-K-F	14.69983	49.96944	42.55087	33.55222	33.94288	49.96944
59	N-P-I-Y-S-N-K-F-G-K-F-F-E-I-T	1.463255	4.739266	1.550267	0.884117	5.243798	5.243798
60	S-N-K-F-G-K-F-F-E-I-T-P-E-K-N	0.521625	-0.3088	0.349762	0.035659	1.346119	2.058859
61	G-K-F-F-E-I-T-P-E-K-N-Q-Q-L-Q	0.70891	0.221433	0.983684	0.408485	2.027549	2.027549
62	E-I-T-P-E-K-N-Q-Q-L-Q-D-L-D-I	0.618736	0.586071	0.616799	-0.01512	2.836668	2.836668
63	E-K-N-Q-Q-L-Q-D-L-D-I-F-V-N-S	0.604863	1.884049	1.740675	0.383713	2.475093	2.475093
64	Q-L-Q-D-L-D-I-F-V-N-S-V-D-I-K	1.222212	2.725393	1.582775	0.5757	2.013643	2.725393
65	L-D-I-F-V-N-S-V-D-I-K-E-G-S-L	1.567302	-0.12481	1.013871	-0.19968	1.241187	1.582683
66	V-N-S-V-D-I-K-E-G-S-L-L-L-P-N	2.541881	0.226451	1.132296	-0.01265	3.267777	3.267777
67	D-I-K-E-G-S-L-L-L-P-N-Y-N-S-R	2.441302	2.885967	2.147036	0.702039	4.686264	4.686264
68	G-S-L-L-L-P-N-Y-N-S-R-A-I-V-I	2.774254	7.502486	4.489994	2.691271	4.586389	7.502486
69	L-P-N-Y-N-S-R-A-I-V-I-V-T-V-T	3.929181	8.002609	6.185098	5.844809	7.312109	8.002609
70	N-S-R-A-I-V-I-V-T-V-T-E-G-K-G	4.185832	9.998082	6.879394	4.547969	5.427114	9.998082
71	I-V-I-V-T-V-T-E-G-K-G-D-F-E-L	2.564425	0.221433	2.258495	0.279668	2.624275	2.624275
72	T-V-T-E-G-K-G-D-F-E-L-V-G-Q-R	3.075992	-2.30594	1.00226	1.019127	3.004814	3.075992
73	G-K-G-D-F-E-L-V-G-Q-R-N-E-N-Q	0.686367	1.870668	0.438	3.061619	5.582617	5.582617
74	F-E-L-V-G-Q-R-N-E-N-Q-G-K-E-N	0.922208	1.977718	0.20115	1.234648	2.794948	2.794948
75	G-Q-R-N-E-N-Q-G-K-E-N-D-K-E-E	0.722783	0.084276	0.275456	0.95348	3.926451	3.926451
76	E-N-Q-G-K-E-N-D-K-E-E-E-Q-E-E	0.998509	0.311757	0.159353	0.517484	2.887238	2.887238
77	K-E-N-D-K-E-E-E-Q-E-E-E-T-S-K	0.760934	-0.46603	-0.02641	0.475371	2.372689	2.372689
78	K-E-E-E-Q-E-E-E-T-S-K-Q-V-Q-L	0.70024	-0.43926	-0.33525	0.827141	2.40556	2.40556
79	Q-E-E-E-T-S-K-Q-V-Q-L-Y-R-A-K	1.801409	2.206871	0.749156	1.033991	2.9947	3.610613
80	T-S-K-Q-V-Q-L-Y-R-A-K-L-S-P-G	3.287555	10.26069	4.81276	5.805173	9.267902	10.26069
81	V-Q-L-Y-R-A-K-L-S-P-G-D-V-F-V	3.282352	8.397355	5.372377	4.929465	9.764752	9.764752
82	R-A-K-L-S-P-G-D-V-F-V-I-P-A-G	0.67076	2.047969	1.2275	0.682221	2.716565	3.148129
83	S-P-G-D-V-F-V-I-P-A-G-H-P-V-A	0.792148	-0.03448	0.312609	0.106261	0.002223	0.792148
84	V-F-V-I-P-A-G-H-P-V-A-I-N-A-S	0.382895	1.576281	1.612962	1.032752	1.682409	1.995344

85	P-A-G-H-P-V-A-I-N-A-S-S-D-L-N	0.840704	2.310575	3.398626	0.570745	2.963093	3.398626
86	P-V-A-I-N-A-S-S-D-L-N-L-I-G-F	0.969029	2.190144	3.700494	0.415917	3.228586	3.700494
87	N-A-S-S-D-L-N-L-I-G-F-G-I-N-A	1.295045	1.547846	4.705946	0.650017	2.558534	4.705946
88	D-L-N-L-I-G-F-G-I-N-A-E-N-N-E	1.634933	1.1531	2.409429	-0.05476	1.212109	2.409429
89	I-G-F-G-I-N-A-E-N-N-E-R-N-F-L	1.952278	1.245096	1.673336	-0.03742	2.950451	2.950451
90	I-N-A-E-N-N-E-R-N-F-L-A-G-E-E	2.295635	-0.35229	1.489893	0.216499	13.70036	13.70036
91	N-N-E-R-N-F-L-A-G-E-E-D-N-V-I	2.188119	-0.05288	2.695042	0.075295	3.76842	3.76842
92	N-F-L-A-G-E-E-D-N-V-I-S-Q-V-E	2.793329	0.273286	1.7012	0.140942	1.562306	2.793329
93	G-E-E-D-N-V-I-S-Q-V-E-R-P-V-K	3.797388	1.420725	2.237596	0.843243	3.61671	3.797388
94	N-V-I-S-Q-V-E-R-P-V-K-E-L-A-F	3.620507	0.766718	2.21902	0.63887	3.997249	3.997249
95	Q-V-E-R-P-V-K-E-L-A-F-P-G-S-S	2.997956	-0.68514	2.160968	1.333738	5.95557	5.95557
96	P-V-K-E-L-A-F-P-G-S-S-H-E-V-D	3.289289	-2.15875	1.262331	0.562075	2.810119	3.289289
97	L-A-F-P-G-S-S-H-E-V-D-R-L-L-K	0.856311	3.489795	0.523916	1.744961	3.376503	3.489795
98	G-S-S-H-E-V-D-R-L-L-K-N-Q-K-Q	0.917005	2.962909	0.231337	1.25075	4.467549	4.467549
99	E-V-D-R-L-L-K-N-Q-K-Q-S-Y-F-A	2.472516	3.079995	2.172579	1.386999	7.477726	7.477726
100	L-L-K-N-Q-K-Q-S-Y-F-A-N-A-Q-P	1.019319	1.646533	0.198828	1.15166	3.66728	3.66728
101	Q-K-Q-S-Y-F-A-N-A-Q-P-L-Q-R-E	0.814692	-0.40247	-0.11929	1.062479	3.298119	3.298119
	m (blanks)	9544.2	12428.62	9019.374	10125.21	10837.24	
	s (blanks)	576.6596	597.8531	430.6522	807.3471	790.9836	

Table A47: Calculated Z-scores of Pis s 1 IgE inhibition experiments.

The calculated Z-scores of serum pool 1 (pea patients 1, 4, 8) and serum pool 2 (pea patients 3, 10, 11, 13) inhibited (+) and uninhibited (-) are listed. For inhibition 30 μg rPis s 1 was preincubated with both serum pools. As references, uninhibited serum pools preincubated with protein buffer without rPis s 1 were used. Identified candidate diagnostic peptides are highlighted in light blue.

	IgE inhibition experiment →	Pool 1 -	Pool 1 +	Pool 2-	Pool 2 +
Peptide No.	Peptide sequence ↓	Z-score	Z-score	Z-score	Z-score
1	S-R-S-D-Q-E-N-P-F-I-F-K-S-N-R	4.836787	3.552239	6.88609	2.935156
2	Q-E-N-P-F-I-F-K-S-N-R-F-Q-T-L	4.353223	13.07424	13.78663	8.044058
3	F-I-F-K-S-N-R-F-Q-T-L-Y-E-N-E	4.360865	2.799871	9.379283	4.011408
4	S-N-R-F-Q-T-L-Y-E-N-E-N-G-H-I	4.822196	0.959604	5.301167	2.312833
5	Q-T-L-Y-E-N-E-N-G-H-I-R-L-L-Q	5.216134	2.751331	24.67098	10.08528
6	E-N-E-N-G-H-I-R-L-L-Q-K-F-D-K	3.957895	4.129863	7.496566	7.749736
7	G-H-I-R-L-L-Q-K-F-D-K-R-S-K-I	3.33885	2.632409	10.73755	4.870945
8	L-L-Q-K-F-D-K-R-S-K-I-F-E-N-L	3.404854	0.896503	5.595652	6.802342
9	F-D-K-R-S-K-I-F-E-N-L-Q-N-Y-R	1.916644	0.949897	5.314402	6.234198
10	S-K-I-F-E-N-L-Q-N-Y-R-L-L-E-Y	2.38006	5.322123	9.63737	7.967915
11	E-N-L-Q-N-Y-R-L-L-E-Y-K-S-K-P	3.626487	2.06874	6.755392	2.169333
12	N-Y-R-L-L-E-Y-K-S-K-P-H-T-L-F	1.44767	1.145876	2.759996	1.946761
13	L-E-Y-K-S-K-P-H-T-L-F-L-P-Q-Y	1.38653	1.540262	8.075609	1.129688
14	S-K-P-H-T-L-F-L-P-Q-Y-T-D-A-D	1.304547	0.380767	7.48664	2.652548
15	T-L-F-L-P-Q-Y-T-D-A-D-F-I-L-V	2.045178	1.941323	32.81232	21.48476
16	P-Q-Y-T-D-A-D-F-I-L-V-V-L-S-G	1.382362	6.946387	14.6039	10.0106
17	D-A-D-F-I-L-V-V-L-S-G-K-A-T-L	1.632481	8.978993	27.75314	19.0189

18	I-L-V-V-L-S-G-K-A-T-L-T-V-L-K	3.27632	3.805253	36.39825	21.2988
19	L-S-G-K-A-T-L-T-V-L-K-S-N-D-R	1.004403	2.276854	12.46475	5.398089
20	A-T-L-T-V-L-K-S-N-D-R-N-S-F-N	0.101195	0.513038	7.003553	2.022904
21	V-L-K-S-N-D-R-N-S-F-N-L-E-R-G	0.156082	-0.22537	6.73223	4.425801
22	N-D-R-N-S-F-N-L-E-R-G-D-A-I-K	0.322828	-0.49598	13.65262	1.167759
23	S-F-N-L-E-R-G-D-A-I-K-L-P-A-G	0.338114	0.072539	11.14784	0.77533
24	E-R-G-D-A-I-K-L-P-A-G-T-I-A-Y	0.579895	-0.94801	5.676718	-1.04478
25	A-I-K-L-P-A-G-T-I-A-Y-L-A-N-R	5.141099	11.70481	8.998769	5.310232
26	P-A-G-T-I-A-Y-L-A-N-R-D-D-N-E	5.249484	5.613362	10.6631	10.23756
27	I-A-Y-L-A-N-R-D-D-N-E-D-L-R-V	7.885463	7.084726	18.61253	10.6256
28	A-N-R-D-D-N-E-D-L-R-V-L-D-L-A	5.912994	8.293064	26.8945	12.14407
29	D-N-E-D-L-R-V-L-D-L-A-I-P-V-N	6.506333	13.22289	38.30578	48.60778
30	L-R-V-L-D-L-A-I-P-V-N-K-P-G-Q	7.010045	6.331751	11.13626	8.244666
31	D-L-A-I-P-V-N-K-P-G-Q-L-Q-S-F	7.774993	7.332886	29.10313	13.79725
32	P-V-N-K-P-G-Q-L-Q-S-F-L-L-S-G	4.041963	2.150044	9.164209	4.286694
33	P-G-Q-L-Q-S-F-L-L-S-G-T-Q-N-Q	4.747855	4.496339	8.527262	31.48439
34	Q-S-F-L-L-S-G-T-Q-N-Q-P-S-L-L	5.086906	3.653566	14.32431	6.619306
35	L-S-G-T-Q-N-Q-P-S-L-L-S-G-F-S	6.309017	2.397597	6.108518	4.589802
36	Q-N-Q-P-S-L-L-S-G-F-S-K-N-I-L	2.591966	0.080427	2.348049	2.033154
37	S-L-L-S-G-F-S-K-N-I-L-E-A-A-F	2.091033	0.223619	7.643809	2.33919
38	G-F-S-K-N-I-L-E-A-A-F-N-T-N-Y	3.239497	1.226574	12.8188	6.036519
39	N-I-L-E-A-A-F-N-T-N-Y-E-E-I-E	2.059073	1.006324	18.61253	11.91417
40	A-A-F-N-T-N-Y-E-E-I-E-K-V-L-L	2.00627	0.585848	13.75188	7.083485
41	T-N-Y-E-E-I-E-K-V-L-L-E-Q-Q-E	0.745252	4.003659	13.66254	6.260555
42	E-I-E-K-V-L-L-E-Q-Q-E-Q-E-P-Q	1.40251	6.180671	48.53829	20.04683
43	V-L-L-E-Q-Q-E-Q-E-P-Q-H-R-R-S	0.348535	-0.21749	8.219543	2.132726
44	Q-Q-E-Q-E-P-Q-H-R-R-S-L-K-D-R	0.345756	-0.53239	9.710164	2.525155
45	E-P-Q-H-R-R-S-L-K-D-R-R-Q-E-I	0.953685	0.965065	12.59049	4.065586
46	R-R-S-L-K-D-R-R-Q-E-I-N-E-E-N	-0.0322	0.695669	11.49858	3.438871
47	K-D-R-R-Q-E-I-N-E-E-N-V-I-V-K	0.7522	-0.65617	5.552637	2.601298
48	Q-E-I-N-E-E-N-V-I-V-K-V-S-R-E	1.661661	-0.97714	5.496387	0.650866
49	E-E-N-V-I-V-K-V-S-R-E-Q-I-E-E	9.235412	8.060376	8.257594	3.78005
50	I-V-K-V-S-R-E-Q-I-E-E-L-S-K-N	9.177746	9.08487	19.15849	6.090698
51	S-R-E-Q-I-E-E-L-S-K-N-A-K-S-S	7.700652	8.1344	14.0596	10.51138
52	I-E-E-L-S-K-N-A-K-S-S-S-K-K-S	7.413015	10.70974	29.87409	23.97259
53	S-K-N-A-K-S-S-S-K-K-S-V-S-S-E	7.622838	10.47554	51.27055	34.37856
54	K-S-S-S-K-K-S-V-S-S-E-S-G-P-F	6.11309	4.186897	14.27302	7.915201
55	K-K-S-V-S-S-E-S-G-P-F-N-L-R-S	8.828274	2.251978	19.27264	7.516915
56	S-S-E-S-G-P-F-N-L-R-S-R-N-P-I	10.49782	3.23855	15.29214	10.72956
57	G-P-F-N-L-R-S-R-N-P-I-Y-S-N-K	11.29403	6.016242	25.00351	12.47646
58	L-R-S-R-N-P-I-Y-S-N-K-F-G-K-F	15.6079	3.832556	19.29249	17.88261
59	N-P-I-Y-S-N-K-F-G-K-F-F-E-I-T	9.659225	5.431944	10.55557	5.468375
60	S-N-K-F-G-K-F-F-E-I-T-P-E-K-N	9.091594	0.958998	9.9302	2.468048
61	G-K-F-F-E-I-T-P-E-K-N-Q-Q-L-Q	5.753196	-0.0482	7.013479	1.391796
62	E-I-T-P-E-K-N-Q-Q-L-Q-D-L-D-I	6.263856	-0.6859	8.509064	0.338972

63	E-K-N-Q-Q-L-Q-D-L-D-I-F-V-N-S	8.295381	3.605633	18.19728	4.737695
64	Q-L-Q-D-L-D-I-F-V-N-S-V-D-I-K	7.484577	3.176055	13.42596	5.253124
65	L-D-I-F-V-N-S-V-D-I-K-E-G-S-L	2.977567	1.065179	15.51548	3.41105
66	V-N-S-V-D-I-K-E-G-S-L-L-L-P-N	4.471335	1.176214	10.27432	5.332196
67	D-I-K-E-G-S-L-L-L-P-N-Y-N-S-R	3.349272	-0.13194	13.14306	2.678905
68	G-S-L-L-L-P-N-Y-N-S-R-A-I-V-I	4.806217	2.89331	13.0438	5.575268
69	L-P-N-Y-N-S-R-A-I-V-I-V-T-V-T	1.187824	4.971422	15.95225	7.389521
70	N-S-R-A-I-V-I-V-T-V-T-E-G-K-G	4.323347	9.239895	13.44085	10.76617
71	I-V-I-V-T-V-T-E-G-K-G-D-F-E-L	3.052603	-1.14824	6.781862	0.70358
72	T-V-T-E-G-K-G-D-F-E-L-V-G-Q-R	8.538552	-1.23622	7.986271	-0.24967
73	G-K-G-D-F-E-L-V-G-Q-R-N-E-N-Q	20.77842	4.721442	7.683514	4.827017
74	F-E-L-V-G-Q-R-N-E-N-Q-G-K-E-N	14.06966	7.043467	9.189026	7.882987
75	G-Q-R-N-E-N-Q-G-K-E-N-D-K-E-E	9.798181	4.054019	27.83007	13.64789
76	E-N-Q-G-K-E-N-D-K-E-E-E-Q-E-E	7.146221	4.13411	38.88151	11.76774
77	K-E-N-D-K-E-E-E-Q-E-E-E-T-S-K	9.278488	3.732443	40.90154	8.695667
78	K-E-E-E-Q-E-E-E-T-S-K-Q-V-Q-L	11.52956	8.86189	36.86313	12.30367
79	Q-E-E-E-T-S-K-Q-V-Q-L-Y-R-A-K	34.76773	1.519026	15.68589	4.324766
80	T-S-K-Q-V-Q-L-Y-R-A-K-L-S-P-G	29.13101	13.58815	19.75407	10.07796
81	V-Q-L-Y-R-A-K-L-S-P-G-D-V-F-V	35.29715	24.24963	13.56163	7.415879
82	R-A-K-L-S-P-G-D-V-F-V-I-P-A-G	35.33258	7.96451	34.07298	6.286912
83	S-P-G-D-V-F-V-I-P-A-G-H-P-V-A	35.27561	4.53153	44.13674	4.37748
84	V-F-V-I-P-A-G-H-P-V-A-I-N-A-S	34.35781	10.50011	6.565135	1.61876
85	P-A-G-H-P-V-A-I-N-A-S-S-D-L-N	10.89454	0.148382	2.925437	1.110652
86	P-V-A-I-N-A-S-S-D-L-N-L-I-G-F	26.15807	4.587351	42.05136	5.518161
87	N-A-S-S-D-L-N-L-I-G-F-G-I-N-A	34.31682	10.38786	25.66197	6.172698
88	D-L-N-L-I-G-F-G-I-N-A-E-N-N-E	34.5718	20.56242	19.84176	5.125731
89	I-G-F-G-I-N-A-E-N-N-E-R-N-F-L	18.42208	1.157404	9.245275	5.250196
90	I-N-A-E-N-N-E-R-N-F-L-A-G-E-E	24.48227	0.85039	20.98165	5.800768
91	N-N-E-R-N-F-L-A-G-E-E-D-N-V-I	33.78809	7.281919	71.46756	5.513768
92	N-F-L-A-G-E-E-D-N-V-I-S-Q-V-E	18.61662	-0.66163	25.073	3.668764
93	G-E-E-D-N-V-I-S-Q-V-E-R-P-V-K	2.072274	-0.81574	9.516598	2.595441
94	N-V-I-S-Q-V-E-R-P-V-K-E-L-A-F	9.355608	-0.965	12.83369	3.993836
95	Q-V-E-R-P-V-K-E-L-A-F-P-G-S-S	34.12784	0.499689	83.10797	2.183976
96	P-V-K-E-L-A-F-P-G-S-S-H-E-V-D	34.31473	-1.10091	80.55026	0.671366
97	L-A-F-P-G-S-S-H-E-V-D-R-L-L-K	34.99214	2.031121	26.60663	1.497224
98	G-S-S-H-E-V-D-R-L-L-K-N-Q-K-Q	13.88485	2.604498	7.673588	2.091726
99	E-V-D-R-L-L-K-N-Q-K-Q-S-Y-F-A	14.77034	2.313866	22.15628	1.56751
100	L-L-K-N-Q-K-Q-S-Y-F-A-N-A-Q-P	30.17387	5.913095	81.90687	1.26001
101	Q-K-Q-S-Y-F-A-N-A-Q-P-L-Q-R-E	33.43723	2.741623	71.95726	1.028652
	m (blanks)	11292.35	13142.45	12164.73	10943.51
	s (blanks)	1439.313	1648.131	604.4458	682.9255

7.1.6 Peptide sequences of PA1, PA2, Gly m 5.03 and Gly m 8

Table A48: Peptide sequences of PA1.

Peptide No.	Peptide sequence
1	A-S-C-N-G-V-C-S-P-F-E-M-P-P-C
2	G-V-C-S-P-F-E-M-P-P-C-G-S-S-A
3	P-F-E-M-P-P-C-G-S-S-A-C-R-C-I
4	P-P-C-G-S-S-A-C-R-C-I-P-V-G-L
5	S-S-A-C-R-C-I-P-V-G-L-V-V-G-Y
6	R-C-I-P-V-G-L-V-V-G-Y-C-R-H-P
7	V-G-L-V-V-G-Y-C-R-H-P-S-G-V-F
8	V-G-Y-C-R-H-P-S-G-V-F-L-R-T-N
9	R-H-P-S-G-V-F-L-R-T-N-D-E-H-P
10	G-V-F-L-R-T-N-D-E-H-P-N-L-C-E
11	R-T-N-D-E-H-P-N-L-C-E-S-D-A-D
12	E-H-P-N-L-C-E-S-D-A-D-C-R-K-K
13	L-C-E-S-D-A-D-C-R-K-K-G-S-G-N
14	D-A-D-C-R-K-K-G-S-G-N-F-C-G-H
15	R-K-K-G-S-G-N-F-C-G-H-Y-P-N-P
16	S-G-N-F-C-G-H-Y-P-N-P-D-I-E-Y
17	C-G-H-Y-P-N-P-D-I-E-Y-G-W-C-F
18	P-N-P-D-I-E-Y-G-W-C-F-A-S-K-S
19	I-E-Y-G-W-C-F-A-S-K-S-E-A-E-D
20	W-C-F-A-S-K-S-E-A-E-D-F-F-S-K
21	S-K-S-E-A-E-D-F-F-S-K-I-T-Q-K
22	A-E-D-F-F-S-K-I-T-Q-K-D-L-L-K
23	F-S-K-I-T-Q-K-D-L-L-K-S-V-S-T
24	S-K-I-T-Q-K-D-L-L-K-S-V-S-T-A

Table A49: Peptide sequences of PA2.

Peptide No.	Peptide sequence
1	M-T-K-T-G-Y-I-N-A-A-F-R-S-S-Q
2	G-Y-I-N-A-A-F-R-S-S-Q-N-N-E-A
3	A-A-F-R-S-S-Q-N-N-E-A-Y-L-F-I
4	S-S-Q-N-N-E-A-Y-L-F-I-N-D-K-Y
5	N-E-A-Y-L-F-I-N-D-K-Y-V-L-L-D
6	L-F-I-N-D-K-Y-V-L-L-D-Y-A-P-G
7	D-K-Y-V-L-L-D-Y-A-P-G-T-S-N-D
8	L-L-D-Y-A-P-G-T-S-N-D-K-V-L-Y
9	A-P-G-T-S-N-D-K-V-L-Y-G-P-T-P
10	S-N-D-K-V-L-Y-G-P-T-P-V-R-D-G
11	V-L-Y-G-P-T-P-V-R-D-G-F-K-S-L
12	P-T-P-V-R-D-G-F-K-S-L-N-Q-T-V

13	R-D-G-F-K-S-L-N-Q-T-V-F-G-S-Y
14	K-S-L-N-Q-T-V-F-G-S-Y-G-V-D-C
15	Q-T-V-F-G-S-Y-G-V-D-C-S-F-D-T
16	G-S-Y-G-V-D-C-S-F-D-T-D-N-D-E
17	V-D-C-S-F-D-T-D-N-D-E-A-F-I-F
18	F-D-T-D-N-D-E-A-F-I-F-Y-E-K-F
19	N-D-E-A-F-I-F-Y-E-K-F-C-A-L-I
20	F-I-F-Y-E-K-F-C-A-L-I-D-Y-A-P
21	E-K-F-C-A-L-I-D-Y-A-P-H-S-N-K
22	A-L-I-D-Y-A-P-H-S-N-K-D-K-I-I
23	Y-A-P-H-S-N-K-D-K-I-I-L-G-P-K
24	S-N-K-D-K-I-I-L-G-P-K-K-I-A-D
25	K-I-I-L-G-P-K-K-I-A-D-M-F-P-F
26	G-P-K-K-I-A-D-M-F-P-F-F-E-G-T
27	I-A-D-M-F-P-F-F-E-G-T-V-F-E-N
28	F-P-F-F-E-G-T-V-F-E-N-G-I-D-A
29	E-G-T-V-F-E-N-G-I-D-A-A-Y-R-S
30	F-E-N-G-I-D-A-A-Y-R-S-T-R-G-K
31	I-D-A-A-Y-R-S-T-R-G-K-E-V-Y-L
32	Y-R-S-T-R-G-K-E-V-Y-L-F-K-G-D
33	R-G-K-E-V-Y-L-F-K-G-D-Q-Y-A-R
34	V-Y-L-F-K-G-D-Q-Y-A-R-I-D-Y-E
35	K-G-D-Q-Y-A-R-I-D-Y-E-T-N-S-M
36	Y-A-R-I-D-Y-E-T-N-S-M-V-N-K-E
37	D-Y-E-T-N-S-M-V-N-K-E-I-K-S-I
38	N-S-M-V-N-K-E-I-K-S-I-R-N-G-F
39	N-K-E-I-K-S-I-R-N-G-F-P-C-F-R
40	K-S-I-R-N-G-F-P-C-F-R-N-T-I-F
41	N-G-F-P-C-F-R-N-T-I-F-E-S-G-T
42	C-F-R-N-T-I-F-E-S-G-T-D-A-A-F
43	T-I-F-E-S-G-T-D-A-A-F-A-S-H-K
44	S-G-T-D-A-A-F-A-S-H-K-T-N-E-V
45	A-A-F-A-S-H-K-T-N-E-V-Y-F-F-K
46	S-H-K-T-N-E-V-Y-F-F-K-G-D-Y-Y
47	N-E-V-Y-F-F-K-G-D-Y-Y-A-R-V-T
48	F-F-K-G-D-Y-Y-A-R-V-T-V-T-P-G
49	D-Y-Y-A-R-V-T-V-T-P-G-A-T-D-D
50	R-V-T-V-T-P-G-A-T-D-D-Q-I-M-D
51	T-P-G-A-T-D-D-Q-I-M-D-G-V-R-K
52	T-D-D-Q-I-M-D-G-V-R-K-T-L-D-Y
53	I-M-D-G-V-R-K-T-L-D-Y-W-P-S-L
54	V-R-K-T-L-D-Y-W-P-S-L-R-G-I-I
55	L-D-Y-W-P-S-L-R-G-I-I-P-L-E-N

Table A50: Peptide sequences of Gly m 5.0301 and Gly m 5.0302.

Gly m 5.0301 contains a leucine (L) in peptides 1-4 and in peptides 41-44. In contrast Gly m 5.0302 contains in the respective peptides a phenylalanine (F). Amino acid differences between both isoforms are shown in red.

Peptide No.	Peptide sequence
1	L-K-V-R-E-D-E-N-N-P-F-Y-**F/L**-R-S
2	E-D-E-N-N-P-F-Y-**F/L**-R-S-S-N-S-F
3	N-P-F-Y-**F/L**-R-S-S-N-S-F-Q-T-L-F
4	**F/L**-R-S-S-N-S-F-Q-T-L-F-E-N-Q-N
5	N-S-F-Q-T-L-F-E-N-Q-N-G-R-I-R
6	T-L-F-E-N-Q-N-G-R-I-R-L-L-Q-R
7	N-Q-N-G-R-I-R-L-L-Q-R-F-N-K-R
8	R-I-R-L-L-Q-R-F-N-K-R-S-P-Q-L
9	L-Q-R-F-N-K-R-S-P-Q-L-E-N-L-R
10	N-K-R-S-P-Q-L-E-N-L-R-D-Y-R-I
11	P-Q-L-E-N-L-R-D-Y-R-I-V-Q-F-Q
12	N-L-R-D-Y-R-I-V-Q-F-Q-S-K-P-N
13	Y-R-I-V-Q-F-Q-S-K-P-N-T-I-L-L
14	Q-F-Q-S-K-P-N-T-I-L-L-P-H-H-A
15	K-P-N-T-I-L-L-P-H-H-A-D-A-D-F
16	I-L-L-P-H-H-A-D-A-D-F-L-L-F-V
17	H-H-A-D-A-D-F-L-L-F-V-L-S-G-R
18	A-D-F-L-L-F-V-L-S-G-R-A-I-L-T
19	L-F-V-L-S-G-R-A-I-L-T-L-V-N-N
20	S-G-R-A-I-L-T-L-V-N-N-D-D-R-D
21	I-L-T-L-V-N-N-D-D-R-D-S-Y-N-L
22	V-N-N-D-D-R-D-S-Y-N-L-H-P-G-D
23	D-R-D-S-Y-N-L-H-P-G-D-A-Q-R-I
24	Y-N-L-H-P-G-D-A-Q-R-I-P-A-G-T
25	P-G-D-A-Q-R-I-P-A-G-T-T-Y-Y-L
26	Q-R-I-P-A-G-T-T-Y-Y-L-V-N-P-H
27	A-G-T-T-Y-Y-L-V-N-P-H-D-H-Q-N
28	Y-Y-L-V-N-P-H-D-H-Q-N-L-K-I-I
29	N-P-H-D-H-Q-N-L-K-I-I-K-L-A-I
30	H-Q-N-L-K-I-I-K-L-A-I-P-V-N-K
31	K-I-I-K-L-A-I-P-V-N-K-P-G-R-Y
32	L-A-I-P-V-N-K-P-G-R-Y-D-D-F-F
33	V-N-K-P-G-R-Y-D-D-F-F-L-S-S-T
34	G-R-Y-D-D-F-F-L-S-S-T-Q-A-Q-Q
35	D-F-F-L-S-S-T-Q-A-Q-Q-S-Y-L-Q
36	S-S-T-Q-A-Q-Q-S-Y-L-Q-G-F-S-H
37	A-Q-Q-S-Y-L-Q-G-F-S-H-N-I-L-E
38	Y-L-Q-G-F-S-H-N-I-L-E-T-S-F-H
39	F-S-H-N-I-L-E-T-S-F-H-S-E-F-E
40	I-L-E-T-S-F-H-S-E-F-E-E-I-N-R

41	S-F-H-S-E-F-E-E-I-N-R-V-L-**F/L**-G
42	E-F-E-E-I-N-R-V-L-**F/L**-G-E-E-E-E
43	I-N-R-V-L-**F/L**-G-E-E-E-E-Q-R-Q-Q
44	L-**F/L**-G-E-E-E-E-Q-R-Q-Q-E-G-V-I
45	E-E-E-Q-R-Q-Q-E-G-V-I-V-E-L-S
46	R-Q-Q-E-G-V-I-V-E-L-S-K-E-Q-I
47	G-V-I-V-E-L-S-K-E-Q-I-R-Q-L-S
48	E-L-S-K-E-Q-I-R-Q-L-S-R-R-A-K
49	E-Q-I-R-Q-L-S-R-R-A-K-S-S-S-R
50	Q-L-S-R-R-A-K-S-S-S-R-K-T-I-S
51	R-A-K-S-S-S-R-K-T-I-S-S-E-D-E
52	S-S-R-K-T-I-S-S-E-D-E-P-F-N-L
53	T-I-S-S-E-D-E-P-F-N-L-R-S-R-N
54	E-D-E-P-F-N-L-R-S-R-N-P-I-Y-S
55	F-N-L-R-S-R-N-P-I-Y-S-N-N-F-G
56	S-R-N-P-I-Y-S-N-N-F-G-K-F-F-E
57	I-Y-S-N-N-F-G-K-F-F-E-I-T-P-E
58	N-F-G-K-F-F-E-I-T-P-E-K-N-P-Q
59	F-F-E-I-T-P-E-K-N-P-Q-L-R-D-L
60	T-P-E-K-N-P-Q-L-R-D-L-D-I-F-L
61	N-P-Q-L-R-D-L-D-I-F-L-S-S-V-D
62	R-D-L-D-I-F-L-S-S-V-D-I-N-E-G
63	I-F-L-S-S-V-D-I-N-E-G-A-L-L-L
64	S-V-D-I-N-E-G-A-L-L-L-P-H-F-N
65	N-E-G-A-L-L-L-P-H-F-N-S-K-A-I
66	L-L-L-P-H-F-N-S-K-A-I-V-I-L-V
67	H-F-N-S-K-A-I-V-I-L-V-I-N-E-G
68	K-A-I-V-I-L-V-I-N-E-G-D-A-N-I
69	I-L-V-I-N-E-G-D-A-N-I-E-L-V-G
70	N-E-G-D-A-N-I-E-L-V-G-I-K-E-Q
71	A-N-I-E-L-V-G-I-K-E-Q-Q-Q-K-Q
72	L-V-G-I-K-E-Q-Q-Q-K-Q-K-Q-E-E
73	K-E-Q-Q-Q-K-Q-K-Q-E-E-E-P-L-E
74	Q-K-Q-K-Q-E-E-E-P-L-E-V-Q-R-Y
75	Q-E-E-E-P-L-E-V-Q-R-Y-R-A-E-L
76	P-L-E-V-Q-R-Y-R-A-E-L-S-E-D-D
77	Q-R-Y-R-A-E-L-S-E-D-D-V-F-V-I
78	A-E-L-S-E-D-D-V-F-V-I-P-A-A-Y
79	E-D-D-V-F-V-I-P-A-A-Y-P-F-V-V
80	F-V-I-P-A-A-Y-P-F-V-V-N-A-T-S
81	A-A-Y-P-F-V-V-N-A-T-S-N-L-N-F
82	F-V-V-N-A-T-S-N-L-N-F-L-A-F-G
83	A-T-S-N-L-N-F-L-A-F-G-I-N-A-E
84	L-N-F-L-A-F-G-I-N-A-E-N-N-Q-R
85	A-F-G-I-N-A-E-N-N-Q-R-N-F-L-A

86	N-A-E-N-N-Q-R-N-F-L-A-G-E-K-D
87	N-Q-R-N-F-L-A-G-E-K-D-N-V-V-R
88	F-L-A-G-E-K-D-N-V-V-R-Q-I-E-R
89	E-K-D-N-V-V-R-Q-I-E-R-Q-V-Q-E
90	V-V-R-Q-I-E-R-Q-V-Q-E-L-A-F-P
91	I-E-R-Q-V-Q-E-L-A-F-P-G-S-A-Q
92	V-Q-E-L-A-F-P-G-S-A-Q-D-V-E-R
93	A-F-P-G-S-A-Q-D-V-E-R-L-L-K-K
94	S-A-Q-D-V-E-R-L-L-K-K-Q-R-E-S
95	V-E-R-L-L-K-K-Q-R-E-S-Y-F-V-D
96	L-K-K-Q-R-E-S-Y-F-V-D-A-Q-P-Q
97	R-E-S-Y-F-V-D-A-Q-P-Q-Q-K-E-E
98	F-V-D-A-Q-P-Q-Q-K-E-E-G-S-K-G
99	Q-P-Q-Q-K-E-E-G-S-K-G-R-K-G-P
100	K-E-E-G-S-K-G-R-K-G-P-F-P-S-I
101	S-K-G-R-K-G-P-F-P-S-I-L-G-A-L
102	K-G-R-K-G-P-F-P-S-I-L-G-A-L-Y

Table A51: Peptide sequence of Gly m 8.

Peptide No.	Peptide sequence
1	S-K-W-Q-H-Q-Q-D-S-C-R-K-Q-L-Q
2	H-Q-Q-D-S-C-R-K-Q-L-Q-G-V-N-L
3	S-C-R-K-Q-L-Q-G-V-N-L-T-P-C-E
4	Q-L-Q-G-V-N-L-T-P-C-E-K-H-I-M
5	V-N-L-T-P-C-E-K-H-I-M-E-K-I-Q
6	P-C-E-K-H-I-M-E-K-I-Q-G-R-G-D
7	H-I-M-E-K-I-Q-G-R-G-D-D-D-D-D
8	K-I-Q-G-R-G-D-D-D-D-D-D-D-D-D
9	R-G-D-D-D-D-D-D-D-D-D-N-H-I-L
10	D-D-D-D-D-D-D-N-H-I-L-R-T-M-R
11	D-D-D-N-H-I-L-R-T-M-R-G-R-I-N
12	H-I-L-R-T-M-R-G-R-I-N-Y-I-R-R
13	T-M-R-G-R-I-N-Y-I-R-R-N-E-G-K
14	R-I-N-Y-I-R-R-N-E-G-K-D-E-D-E
15	I-R-R-N-E-G-K-D-E-D-E-E-E-E-G
16	E-G-K-D-E-D-E-E-E-E-G-H-M-Q-K
17	E-D-E-E-E-E-G-H-M-Q-K-C-C-T-E
18	E-E-G-H-M-Q-K-C-C-T-E-M-S-E-L
19	M-Q-K-C-C-T-E-M-S-E-L-R-S-P-K
20	C-T-E-M-S-E-L-R-S-P-K-C-Q-C-K
21	S-E-L-R-S-P-K-C-Q-C-K-A-L-Q-K
22	S-P-K-C-Q-C-K-A-L-Q-K-I-M-E-N
23	Q-C-K-A-L-Q-K-I-M-E-N-Q-S-E-E
24	L-Q-K-I-M-E-N-Q-S-E-E-L-E-E-K

25	M-E-N-Q-S-E-E-L-E-E-K-Q-K-K-K
26	S-E-E-L-E-E-K-Q-K-K-K-M-E-K-E
27	E-E-K-Q-K-K-K-M-E-K-E-L-I-N-L
28	K-K-K-M-E-K-E-L-I-N-L-A-T-M-C
29	E-K-E-L-I-N-L-A-T-M-C-R-F-G-P
30	I-N-L-A-T-M-C-R-F-G-P-M-I-Q-C
31	T-M-C-R-F-G-P-M-I-Q-C-D-L-S-S
32	C-R-F-G-P-M-I-Q-C-D-L-S-S-D-D

7.1.7 Mass spectrometry analysis

7.1.7.1 Peanut extract

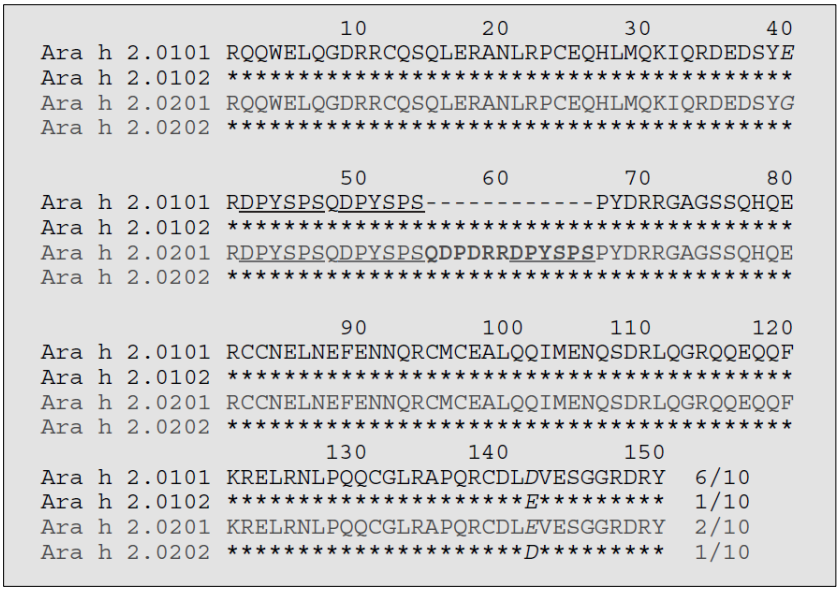


Figure A22: Alignment of variants of nAra h 2.01 and nAra h 2.02 (Hales et al. 2004).

Table A52: Ara h 2.0101- and Ara h 2.0201-specific peptides identified with MS^E.

Band	PM	E	S	Start	End	Peptide sequence	Description
1	1121.5	0.5	8.0	160	169	CDLEVESGGR	Ara h 2.0201
1	1196.5	2.2	6.7	82	91	DPYSPSPYDR	Ara h 2.0201
2	1107.5	0.9	8.6	129	138	CDLDVESGGR	Ara h 2.0101
2	2865.2	6.3	7.1	56	79	DEDSYERDPYSPSQDPYSPSPYDR	Ara h 2.0101
2	1236.5	1.7	6.8	160	170	CDLEVESGGRD	Ara h 2.0201
3	1121.5	0.8	6.8	160	169	CDLEVESGGR	Ara h 2.0201
4	1121.5	5.0	7.2	160	169	CDLEVESGGR	Ara h 2.0201
5	1107.5	1.3	7.8	129	138	CDLDVESGGR	Ara h 2.0101

PM, precursor mass; E, precursor mass error [ppm]; S, PLGS peptide score; start and end position refers to the accession number outlined in Table 23.

7.1.7.2 Pea extract

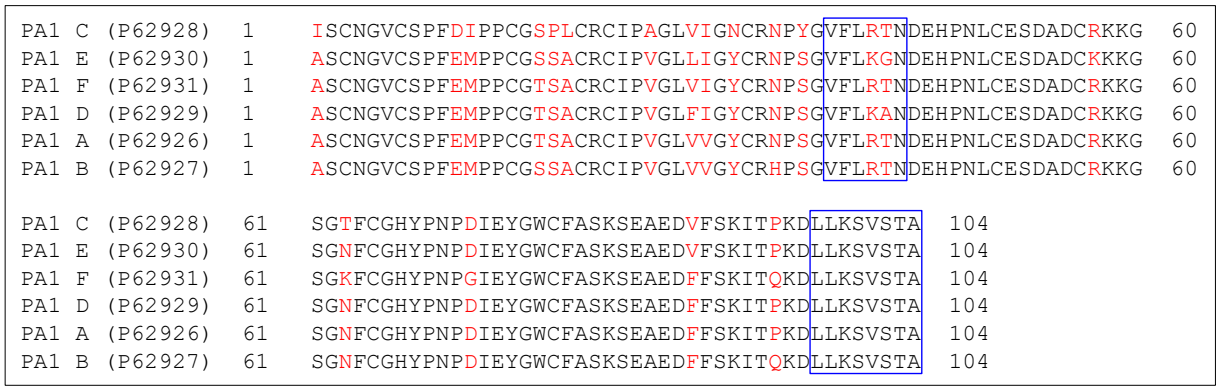


Figure A23: Alignment of PA1 isoforms.

Multiple sequence alignment of PA1 isoforms PA1 A-F. Numbers written in parentheses represent the respective UniProt accession number. Differences in the amino acid sequences are shown in red. Amino acid residues framed in a blue box belong to the propeptide.

Table A53: PA1 isoform-specific peptides identified with MS^E.

Band	PM	E	S	Start	End	Peptide sequence	Description
3	1059.5	0.7	6.0	109	117	SEAEDFFSK	PA1 peptide shared by isoform A, B, D and F
3	1902.7	0.4	6.0	68	83	ANDEHPNLCESDADCR	PA1 D
4	1059.5	-0.5	8.4	109	117	SEAEDFFSK	PA1 peptide shared by isoform A, B, D and F
4	2663.1	-1.1	7.2	86	108	GSGNFCGHYPNPDIEYGWCFASK	PA1 peptide shared by isoform A, B, D and E
4	1902.7	-0.1	7.9	68	83	ANDEHPNLCESDADCR	PA1 D
4	1011.5	-0.1	8.2	109	117	SEAEDVFSK	PA1 peptide shared by isoform E and C

PM, precursor mass; E, precursor mass error [ppm]; S, PLGS peptide score; start and end position refers to the accession number outlined in Table 27; amino acid residues written in blue belong to the propeptide.

7.1.7.3 Soybean extract

Table A54: Gly m 8-specific peptides identified with MS^E.

Band	PM	E	S	Start	End	Peptide sequence	Description
1	1478.7	-1.3	8.4	117	128	IMENQSEELEEK	Gly m 8
1	1220.6	-0.4	7.6	136	145	ELINLATMCR	Gly m 8
1	1386.7	-0.2	7.5	34	45	QLQGVNLTPCEK	Gly m 8
2	1478.7	0.0	9.2	117	128	IMENQSEELEEK	Gly m 8
2	1220.6	0.6	8.5	136	145	ELINLATMCR	Gly m 8
2	1484.6	0.8	8.1	146	158	FGPMIQCDLSSDD	Gly m 8
2	1386.7	0.2	8.0	34	45	QLQGVNLTPCEK	Gly m 8
2	1185.5	1.0	7.9	97	105	CCTEMSELR	Gly m 8
3	1478.7	-0.1	10.0	117	128	IMENQSEELEEK	Gly m 8
3	1220.6	0.3	9.7	136	145	ELINLATMCR	Gly m 8
3	1185.5	0.4	9.7	97	105	CCTEMSELR	Gly m 8
3	1386.7	0.4	9.5	34	45	QLQGVNLTPCEK	Gly m 8
3	1484.6	0.5	9.4	146	158	FGPMIQCDLSSDD	Gly m 8
3	1514.8	-0.4	8.4	33	45	KQLQGVNLTPCEK	Gly m 8
3	1475.6	-1.3	8.4	85	96	DEDEEEEGHMQK	Gly m 8
4	1478.7	0.8	8.9	117	128	IMENQSEELEEK	Gly m 8
4	1386.7	0.2	8.9	34	45	QLQGVNLTPCEK	Gly m 8
4	1484.6	1.5	8.8	146	158	FGPMIQCDLSSDD	Gly m 8
4	1220.6	0.6	8.7	136	145	ELINLATMCR	Gly m 8
4	1185.5	0.4	8.7	97	105	CCTEMSELR	Gly m 8
4	1514.8	-0.2	8.4	33	45	KQLQGVNLTPCEK	Gly m 8
4	1387.7	-0.2	7.8	34	45	QLQGVNLTPCEK	Gly m 8
4	1221.6	0.3	7.6	136	145	ELINLATMCR	Gly m 8
4	1734.8	0.8	7.5	117	130	IMENQSEELEEKQK	Gly m 8
4	1387.7	0.5	7.5	34	45	QLQGVNLTPCEK	Gly m 8
5	1494.7	2.6	7.9	117	128	IMENQSEELEEK	Gly m 8
5	1386.7	-6.4	7.7	34	45	QLQGVNLTPCEK	Gly m 8
5	1201.5	4.4	7.4	97	105	CCTEMSELR	Gly m 8
5	1236.6	-1.1	7.3	136	145	ELINLATMCR	Gly m 8
5	1514.8	2.5	7.2	33	45	KQLQGVNLTPCEK	Gly m 8
5	1500.6	-1.8	7.1	146	158	FGPMIQCDLSSDD	Gly m 8
5	1185.5	-0.7	6.6	97	105	CCTEMSELR	Gly m 8

PM, precursor mass; E, precursor mass error [ppm]; S, PLGS peptide score; start and end position refers to the accession number outlined in Table 35.

7.1.8 Circular dichroism spectroscopy

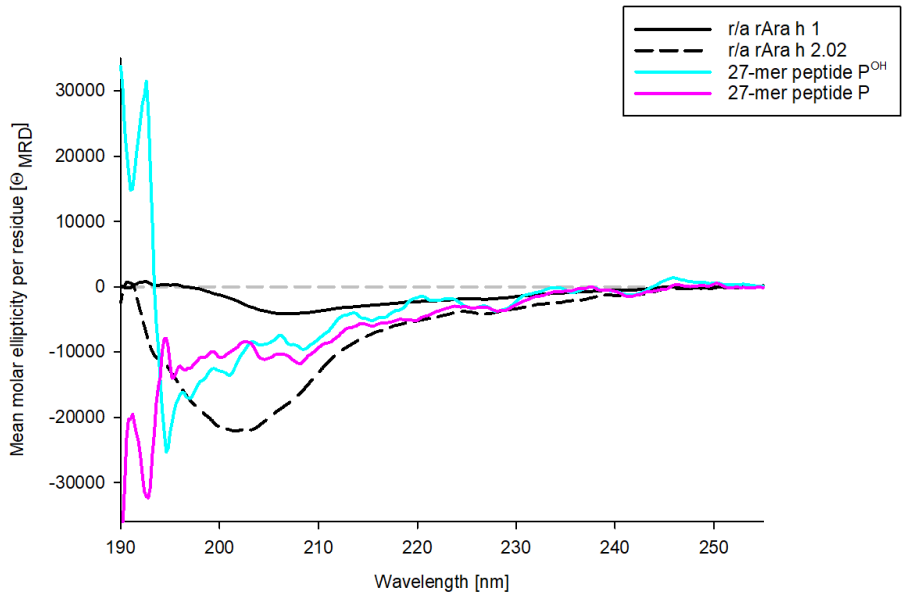


Figure A24: Circular dichroism spectra of r/a rAra h 1, r/a rAra h 2.02 and 27-mer peptides.
Far-UV CD-spectra (190-255nm) of r/a rAra h 1 (black solid line), r/a rAra h 2.02 (black dashed line), 27-mer peptide P (pink) and 27-mer peptide P^{OH} (cyan). Samples were measured at 3 μM (exception r/a rAra h 1 at 2 μM).

7.1.9 IgE immunoblot inhibition

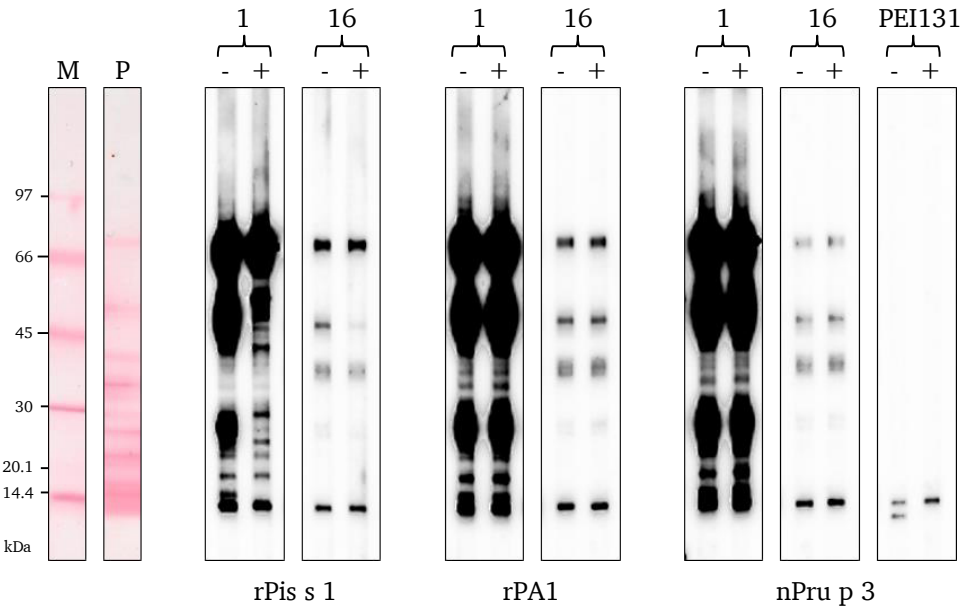


Figure A25: IgE immunoblot inhibition using rPis s 1, rPA1 and nPru p 3.
IgE immunoblot inhibition of pea total protein using sera of pea-allergic patient 1, pea-tolerant patient 16 and an LPT-sensitized donor (PEI131). Serum samples were preincubated with inhibitor protein (+) or untreated (-). As inhibitors rPis s 1, rPA1 and nPru p 3 were used. Pea extract was analyzed under reducing conditions. The detection after 2 min of exposure is shown. M, low-molecular weight marker; P, total protein stained with Ponceau S.

7.2 *Curriculum vitae*

M.Sc.

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Personal data

Birth	July 28th, 1989 in Seligenstadt, Germany
Nationality	German
Marital Status	Married

Professional Experience

01/2019 – present	**Account Manager** at Eppendorf Vertrieb Deutschland GmbH

Education

10/2014 – 11/2017	**Doctorate** at Paul-Ehrlich-Institut in Langen, Germany Division of Allergology Section "Recombinant Allergen Therapeutics" Supervisors: PD Dr. Thomas Holzhauser and Prof. Dr. Harald Kolmar
10/2012 – 07/2014	**Master of Science in Technical Biology**, Technische Universität Darmstadt, Germany Final grade: 1.17 Master thesis: *Analysis of functional IgE epitopes of birch pollen-related allergens* Supervisors: Dr. Dirk Schiller and Prof. Dr. Harald Kolmar
10/2009 – 08/2012	**Bachelor of Science in Biology**, Technische Universität Darmstadt, Germany Final grade: 1.68 Bachelor thesis: *Overexpression of AtPIP1;2 in Nicotiana tabacum* Supervisors: Dr. Marlies Heckwolf and Prof. Dr. Ralf Kaldenhoff

07/2000 – 06/2009 **High school diploma,** Einhardgymnasium Seligenstadt, Germany

Final grade: 2.1

Jasmin Popp

7.3 Danksagung

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8 Affirmations

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Erklärung

Ich erkläre hiermit, dass ich meine Dissertation selbstständig und nur mit den angegebenen Hilfsmitteln angefertigt und noch keinen Promotionsversuch unternommen habe.

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Erklärung der Übereinstimmung

Ich erkläre hiermit, dass die elektronische Version der Doktorarbeit mit der schriftlichen Version übereinstimmt. Die elektronische Version liegt dem Prüfungssekretariat vor.

Jasmin Popp